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Effects of exogenous immunoglobulins in neonatal animals

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Effects of exogenous immunoglobulins in neonatal animals

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CHAPTER ONE

GENERAL INTRODUCTION

Dissertation Organization

The following dissertation is organized into seven chapters. Chapter One is a review of the literature covering immunity in neonatal animals. Chapters Two through Six are a summary of research conducted to examine the effects of administration of exogenous immunoglobulin in neonatal animals. General conclusions from the experiments are in Chapter Seven.

Neonatal Immunity: A Review

Introduction

The transition from fetal to neonatal life is a dramatic event in the life of any animal. Not only is the process of parturition itself demanding, but the fetus must also adapt from a state of maternal reliance to a state of complete independence. There are a number of metabolic and physiologic changes that must occur as the fetus adjusts to life outside the uterus, and the immune system is just one of the systems affected. Prior to parturition, the fetus is sheltered from most antigenic stimuli and is primarily protected by the maternal immune system. After birth, the immune system of the neonate must fend off antigenic challenges unassisted. This review focuses on the developing immune system of neonates, with emphasis on domestic farm animals.

Fetal Immunity

The fetal immune system is often incorrectly assumed to be unable to respond to antigen. Fetal calves (age 195-253 d) are able to mount both cell-mediated and humoral immune responses that are similar to adult cattle in response to tetanus toxoid injection (Tierney and

Simpson-Morgan, 1997). Immunoglobulin M and G can also be detected in the serum of 50 d old pig fetuses after intrauterine injection of antigen (Tlaskalova-Hogenova et al., 1994). The typical age at which lymphoid tissue appears in cattle is in Table 1.1

Table 1.1. Lymphoid tissue development in the fetal calf (Schultz et al., 1971).

Tissue	**Time of appearance (d)**
Thymus	42
Blood lymphocytes	45
Spleen	55
IgM containing cells	59
Peripheral lymph nodes	60
Mesenteric lymph nodes	100
Serum IgM	130
Blood granulocytes	130
IgG containing cells	145
Serum IgG (bacterially infected fetus)	145
GI tract lymphoid tissue	175

The majority of T-cells in fetal lambs are CD5+ and the percentage of γδ T-cells increases 15-38% during the last 20-30 d of fetal life. Overall, the ratio of γδ T-cells to αβ T-cells increases with fetal development (Washington et al., 1992).

In humans, splenic cells are capable of synthesizing immunoglobulin M and G after 19 weeks of gestation, and from 20 weeks of gestation onward, IgM can be detected in the serum of human fetuses (van Furth et al., 1965). However, synthesis of IgA or IgD is not detected up to 30 weeks of gestation, and no immunoglobulin synthesis is detected in the fetal thymus (van Furth et al., 1965). Immunoglobulin A secreting cells are also not found in cattle fetuses (Schultz et al., 1971).

Neonatal Immunity

The human placenta is permeable to and favors fetal uptake of IgG (Dancis et al., 1961; Freda, 1962; Gitlin et al., 1964; Hobbs and Davis, 1967). Newborn babies have concentrations of serum IgG at approximately 89% of adult values, concentrations of serum IgM at 11% of adult values, and concentrations of serum IgA at only 1% of adult values. In contrast to humans, calves, piglets, lambs, and foals are essentially agammaglobulinemic at birth, although a small percentage may have negligible amounts of circulating serum IgG_1, IgG_2, IgM, and IgA. In these domestic farm animals, passive immunity is provided through colostrum (Smith and Holm, 1948; Klaus et al., 1969; Porter, 1969; McGuire and Crawford, 1973; Jeffcott, 1974a; Husband and Lascelles, 1975; Stott et al., 1979c; Edwards et al., 1982; Lavoie et al., 1989; Sheoran et al., 2000).

Bovine colostrum contains high concentrations of IgG, which decrease steadily after the first milking, with only 78% of the initial IgG concentration present at 12 h, and 48% of the initial IgG concentration present at 24 h (Oyeniyi and Hunter, 1978; Levieux and Ollier, 1999). In species lacking significant placental IgG transfer, colostral IgG is absorbed in the small intestine by two main mechanisms: selective (receptor-mediated), or non-selective antibody transfer.

Selective Intestinal Transfer of IgG

Neonatal rats utilize selective (receptor-mediated) IgG transfer in the intestine. The small intestine of one-day-old rats absorbs proteins non-specifically. However, by 3 d of age, IgG absorption is slightly increased compared to albumin and by 7 d of age, the small intestine absorbs IgG 20 times more selectively, with little to no absorption of albumin. At 21 d of age, no proteins are absorbed intact into the circulation (Bangham and Terry, 1957; Jordan

and Morgan, 1968; Jones, 1974). Expression of IgG binding protein also supports this trend, with increased expression in the small intestine from 0-21 d of age, and very low expression by 30 d of age (Jakoi et al., 1985).

The large molecular weight molecule polyvinyl pyrrolidone (PVP; MW 160,000) also exhibits a similar absorption pattern to IgG. More than 50% of ingested PVP can be recovered from intestinal cells of rats younger than 18 d. Between 18-20 d the amount of PVP uptake declines progressively, with less than 5% absorbed after 20 d. This suggests that termination of antibody uptake in rats may be related to the loss of the ability to absorb high molecular weight molecules (Clarke and Hardy, 1969). However, PVP was detected in vacuoles of the distal intestine in this study, which exhibit non-selective protein absorption. This is in contrast to proximal cells, which selectively transport antibodies (Mackenzie, 1972; Rodewald, 1973; Jones, 1976; Mackenzie et al., 1983a; Mackenzie et al., 1983b).

Antibodies from colostrum adhere to localized areas at the base of the microvilli in the proximal portion of the rat small intestine where they are selected to enter the cell; they are absorbed by pinocytosis into tubular vesicles. Antibodies are then transferred to spherical membrane coated vesicles and transferred to the lateral cell membrane where the contents are expelled into the lateral extracellular space (Rodewald, 1970; Rodewald, 1973). The IgG selection process can also be saturated, as IgG molecules compete for binding sites (Jones and Waldmann, 1972). After 22 d of age, proximal rat intestinal cells lose the ability to selectively absorb antibodies as described above. Material that enters cells is degraded instead of transported (Rodewald, 1973).

The IgG binding protein from the proximal portion of rat small intestine preferentially binds IgG_1. Preferential order of affinity is $IgG_1 > IgG_2b > IgG_2a > IgG_3$ (Jakoi et al., 1985).

Optimal binding occurs at a pH of 6.0, with reduced affinity at higher and lower pH (Rodewald, 1976; Mackenzie et al., 1983b; Jakoi et al., 1985). Luminal pH recordings in different segments of intestine in 9-18 d old rats are: stomach, 5.1; duodenum, 6.2; jejunum, 6.3; ileum, 6.9; supporting optimal IgG binding in the duodenum (Rodewald, 1976).

The rat intestine also exhibits some preferences for species-specific IgG. Immunoglobulin G is transported intact across the intestine in neonatal rats with homologous antibody, as well as rabbit, primate, and human antibody being transferred preferentially over heterologous antibody, such as bovine or sheep; however, all IgG types are bound specifically over other proteins (Bangham and Terry, 1957; Bamford, 1966; Jordan and Morgan, 1968; Jones, 1972; Mackenzie, 1972; Jones, 1974; Mackenzie et al., 1983a; Mackenzie et al., 1983b). Also, neonatal rat intestine selectively absorbs sheep IgG_2 in preference to sheep IgG_1 (Mackenzie, 1972).

Non-selective Intestinal Transfer of IgG

In contrast to rats, neonatal domestic farm animals utilize non-selective intestinal transport of IgG molecules. Immunoglobulins G, M, and A are all absorbed non-selectively by neonatal calves in amounts relating to the proportion of the molecules in the colostrum consumed (Bangham et al., 1958; Klaus et al., 1969; Brandon and Lascelles, 1971; Stott et al., 1979b; Besser et al., 1985). In contrast, IgG is preferentially absorbed (compared to IgM and IgA) from colostrum in foals and piglets, and serum IgG concentrations reflect colostral IgG concentration (McGuire and Crawford, 1973; Butler et al., 1981; Morris et al., 1985). The piglet small intestine transports IgG and albumin with equal efficiency when these proteins are given separately; however, IgG transport efficiency is increased when given in combination with increasing amounts of another macromolecule such as albumin or PVP

(Leary and Lecce, 1979). Also in piglets, IgA and IgM from colostrum are found adsorbed to the enterocyte apical surface; however, only faint amounts are detected in cells (Butler et al., 1981).

Unidentified factors in colostrum increase the rate of globulin absorption in neonatal calves. However, these factors are only effective prior to intestinal closure (Balfour and Comline, 1962). Colostrum also enhances PVP absorption from the neonatal foal intestine by 10% (Jeffcot, 1974b).

Concentrations of IgG_1, IgG_2, IgA, and IgM in the serum of calves are strongly correlated to the length of time between birth and suckling (Edwards et al., 1982). The highest rate of IgG absorption occurs during the first four hours after feeding, and absorption rate decreases with increasing age (Kruse, 1970; Stott et al., 1979b; Matte et al., 1982). Fifty percent of calves fed at 24 h of age are unable to absorb IgG, as are 23% of calves fed at 20 h and 10% of calves fed at 16 h (Stott et al., 1979a). In colostrum-fed calves, concentrations of serum gamma globulin increase steadily until approximately 16 h of age, at which time levels plateau (Patt et al., 1972).

Similar to the calf, the small intestine of neonatal foals demonstrates maximum uptake of PVP soon after birth, with peak concentrations attained 6 h after administration. There is a progressive reduction in uptake to less than 1% of the dose entering the circulation at 20 h of age; PVP uptake is 22% of the total dose fed to foals at 3 h compared to 0.9% for foals fed at 20 h (Jeffcott, 1974b).

Mean time to gut closure in calves occurs approximately 26 h after birth for IgG, IgA, and IgM, and the amount of colostrum fed does not affect time to closure (Stott et al., 1979a). Calves older than 48 h of age do not show increases in serum immunoglobulin after infusion

of colostrum into the small intestine (Smith and Erwin, 1959). Age of first feeding can influence closure time in a linear fashion, with earlier feeding resulting in earlier closure. Gut closure to IgG absorption occurs at 21 h if calves are fed at birth and 33 h if feeding is delayed until 24 h. However, when colostrum feeding is delayed, the total absorptive period is decreased (21 h if fed at birth compared to 9 h if fed at 24 h) and maximum serum IgG concentrations are lower (Stott et al., 1979a; Stott et al., 1979c).

Gut closure in domestic farm animals appears to be related to energy (glucose) availability and maturation of the intestine. The small intestine of fetal lambs absorbs up to 20% of IgG infused for sustained periods of time, with no evidence of closure (Smeaton and Simpson-Morgan, 1985). Fetal lambs also have very low circulating glucose levels (Alexander et al., 1955; Comline and Silver, 1970). The ability of intestinal enterocytes to transport macromolecules, such as albumin and egg white, ceases between 24 –36 h postnatally in piglets; however, piglets fasted from birth until 60 h of age retain this transport ability (Leary and Lecce, 1978). Hypoxic calves (pO_2 ~ 26 mm Hg) do not exhibit gut closure until 42-48 h of age, whereas normoxic calves exhibit closure by 24 h of age (Tyler and Ramsey, 1991a). Hypoxia prolongs the time to gut closure by affecting postnatal increases in glucose; peak concentrations of glucose are reached at 42 h instead of 24 h as observed in control calves (Tyler and Ramsey, 1991b).

Intestinal cell maturity and turnover rate also influences gut closure. Fetal lamb small intestinal crypt cells undergo mitosis at a slower rate than neonatal crypt cells, and therefore cell turnover is decreased (Smeaton and Simpson-Morgan, 1985). Epithelial cell migration is more rapid in one-day-old compared to 3-week old lambs, and is more rapid in the proximal compared to the distal small intestine (Moon and Joel, 1975). Cells migrate from the crypts

to approximately half way up the villous in 48 h in both one-day-old lambs and calves, and reach the tips of the villi in approximately 72 h (Moon and Joel, 1975; Smeaton and Simpson-Morgan, 1985).

Prior to colostrum ingestion in newborn lambs, small intestinal epithelial cells stain poorly with basally located nuclei. The nuclei are displaced apically by large protein-filled vacuoles after colostrum ingestion. Cells filled with large vacuoles are visible along the villi the first day after birth, are only along the upper portion by the second day after birth, and are absent by the third day after birth (Smeaton and Simpson-Morgan, 1985).

The ability of enterocytes from the small intestine of the neonatal piglet to take up macromolecules ceases (closure) in a sequential pattern from proximal to distal. Transposition of distal intestinal segments proximally does not affect macromolecule internalization. Thus, cellular differentiation with regard to macromolecule uptake occurs prior to birth, and flow of digesta does not influence time of closure (Leary and Lecce, 1976).

Globulins are absorbed unchanged from the intestine of neonatal calves, and enter the lymphatic system 60-120 min after absorption. Globulins do not enter the portal circulation; instead, they enter peripheral blood from the lymphatic system (Comline et al., 1951; Balfour and Comline, 1961). Once in the circulation, distribution of IgG into the intravascular and extravascular space in newborn foals is 1:1, respectively, with equilibrium being reached in approximately 51 h (Reilly and Macdougall, 1973).

Immunoglobulin G can also be secreted from the circulation back into the intestinal lumen through Fc receptors (FcR) located in the duodenal crypt cells (Besser et al., 1988; Mayer et al., 2002). Antibodies from circulation that appear in the intestinal tract can prevent infection and diarrhea in calves (Besser et al., 1988).

Factors Affecting IgG Absorption

The proximal neonatal piglet small intestine transports more IgG into blood than does the distal small intestine; however, enterocytes from both segments internalize similar amounts of IgG. It appears that there is a difference between the ability of the enterocytes of the two segments to transport macromolecules from within the enterocyte into the blood (Leary and Lecce, 1979).

As observed with rats, the absorptive process in the calf intestine appears to be saturable. Feeding 0.5 or 1 L of colostrum to calves for the first feeding does not saturate the absorptive ability of the gut, since a second feeding at 12 h increases absorption rates. However, feeding 2 L for the first feeding does saturate the absorptive ability of the intestine as no increase is observed after feeding at 12 h (Stott et al., 1979b).

The rate of IgG absorption increases as amount of colostrum fed increases (Stott et al., 1979b; Stott et al., 1979d; Stott an Fellah, 1983). Calves fed larger amounts of colostrum (up to 2 L) at the initial feeding attain higher serum IgG concentrations. However, as age increases, response to initial feeding decreases (Stott et al., 1979c). Method of feeding also appears to have some influence on IgG absorption. Calves that suckle have higher rates of IgG absorption for the first 12 h after birth and attain higher total serum IgG concentrations compared to calves that are bottle fed a similar amount colostrum (Stott et al., 1979d).

Apparent efficiency of absorption (AEA) of IgG_1 and IgM in calves decreases with increasing mass of the antibody in colostrum (Besser et al., 1985). When the mass of IgG, IgA, or IgM is held constant, greater amounts are absorbed when the concentration in the colostrum is higher – for example 1 L of colostrum containing 100 mg IgG/ml has is absorbed with greater efficiency than 2 L of colostrum containing 50 mg IgG/ml. However,

when IgG concentration in colostrum is low (10-20 mg IgG/ml) there is no difference in absorption between feeding 1 L of colostrum containing 20 mg IgG/ml vs. 2 L of colostrum containing 10 mg IgG/ml (Stott and Fellah, 1983).

The AEA of IgG in calves fed colostrum ranges from 23-32% (Morin et al, 1997; Drewry et al., 1999). Efficiency of absorption of IgM is almost 100%. The apparently lower absorptive efficiency for IgG is most likely due to loss of the molecule into the interstitial space (Husband et al., 1972). Efficiency of IgG and IgA absorption in calves does not appear to be influenced by amount of IgG or IgA fed or the concentration in colostrum (Kruse, 1970; Stott and Menefee, 1978; Morin et al, 1997). Calves fed 3.8 L of colostrum in one feeding or divided into two feedings given 10-12 h apart attained similar total serum IgG concentrations and had a similar apparent efficiency of absorption (Hopkins and Quigley et al., 1997). Morin et al. (1997) also reported no difference in IgG_1 concentration in calves fed 2 L three times (0 h, 6 h, and 12 h) and calves fed 4 L at birth and 2 L at 12 h.

Although AEA does not change, the amount of IgG and IgA absorbed by calves by 12 h of age increases positively with the concentration fed (Stott and Fellah, 1983). Immunoglobulin M absorption appears to be slightly different than IgG or IgA, in that as amount of IgM in colostrum increases, serum IgM concentration increases at a decreasing rate (Stott and Menefee, 1978; Stott and Fellah, 1983).

There are a variety of factors that have been suggested to influence colostral absorption of IgG, including the presence of microorganisms, colostral acidity, and presence of steroids. The addition of live microorganisms to the small intestine prior to colostrum ingestion decreased IgG absorption in neonatal calves (James and Polan, 1978; James et al., 1981). Also, addition of potassium isobutyrate to colostrum decreased absorption efficiency of IgG

to 25%, compared to 36% for unaltered colostrum (Baumwart et al., 1977). In contrast, the addition of whey protein concentrate or casein to maternal colostrum had no effect on IgG absorption by neonatal calves (Davenport et al., 2000). Colostral IgG_1 absorption was also not affected by treatment of calves with alkalinizing agents (Ayers and Besser, 1992). Low serum corticosteroid concentration is associated with decreased IgG uptake in neonatal calves; normal or increased corticosteroid concentrations has no effect on IgG uptake (Johnston and Oxender, 1979; James et al., 1981). Elevated levels of serum cortisol also did not affect IgG uptake in foals (Carrick et al., 1987). Hypercapnia as determined at 1 h after birth did not affect AEA of colostrum or plasma IgG concentration at 25 h after birth (Drewry et al., 1999). Added histamine in gamma globulin preparations also did not affect gamma globulin concentrations in the serum of young calves (Patt et al., 1972).

Endogenous IgG Production

The half-life for IgG_2 in the newborn infant is 16 days (Gitlin et al., 1964), and serum IgG levels in newborns decrease rapidly to approximately 37% of adult values by three months of age, and then increase rapidly to two years of age. After this time, serum IgG levels steadily increase until approximately 10 years of age, the time at which adult levels are attained (Stiehm and Fudenberg, 1966). There is no difference between term and preterm infants in the number of IgG and IgA secreting cells after birth, however, premature infants have lower numbers of IgM secreting cells after birth compared to term infants (Stoll et al., 1993).

The concentrations of IgG_1, IgG_2, IgM, and IgA in colostrum deprived calves begin to increase within a few days after birth, reaching levels comparable to colostrum fed calves for IgG_2, IgM, and IgA by 16 - 32 d of age (Husband and Lascelles, 1975; Crowley et al., 1994).

Concentrations of IgG_1 and IgG_2 are reported to increase three-fold during the first eight days of life in colostrum deprived calves (Husband and Lascelles, 1975). In contrast, serum IgG concentrations are undetectable in colostrum-deprived foals until after 7 d of age, and concentrations remain negligible until two weeks of age. After this time, serum IgG concentrations rapidly begin to increase (Jeffcott, 1974a; Holmes and Lunn, 1991).

A transitional period exists when passive immunity is waning and active immunity is developing from two weeks to four months of age in foals. Passively transferred equine IgG has a serum half-life of 23-39 d in newborn foals (Reilly and MacDougall, 1973; Jeffcott, 1974a; Lavoie et al., 1989; Wilson et al., 2001). The half-life for maternally derived influenza antibodies is estimated at 27-39 d depending on the influenza strain and the IgG isotype (Van Maanen et al., 1992; Wilson et al, 2001). Smith and Holm (1948) estimated the half-life for passively acquired IgG in calves at 16-50 d and also reported that half-life depends on the specific antibody. Production of endogenous IgG rapidly increases when concentrations of passively attained IgG become low (Jeffcott, 1974a). More specifically, concentrations of IgG in colostrum-fed foals and calves decrease steadily until approximately four to six weeks of age, after which time IgG concentrations begin increasing again (Boyd, 1972; Wichtel et al., 1991).

Passively acquired antibody also exerts immunosuppressive effects in neonates. Endogenous IgG production in foals fed bovine colostrum is not detected before 28 d of age (Lavoie et al., 1989); whereas, endogenously produced IgG is detected in colostrum-deprived foals after 7 d of age and increases rapidly until reaching levels of 10 g/L by 102-135 d of age (Holmes and Lunn, 1991). Significant endogenous production of IgG and IgM begins around day 8-16 in colostrum-fed calves, while production of IgA isn't detected until day 64.

Concentrations of IgM and IgA are almost undetectable before endogenous production begins (Husband et al., 1972). Colostrum-deprived calves and foals also attain higher levels of endogenous IgG sooner than colostrum-fed animals (Jeffcott, 1974a; Husband and Lascelles, 1975).

Clover and Zarkower (1980) reported a decrease in peripheral blood lymphocyte response to mitogens at 6 and 12 h of age in colostrum-fed calves. However, a similar response was seen in non-stimulated control lymphocytes obtained from calves at the same age, suggesting a general decreased lymphocyte responsiveness at this age. Immunoglobulin G_1 and G_2 positive cells are present throughout the peripheral and mesenteric lymph nodes in colostrum deprived and mid-term fetal calves. In contrast, no IgG_1 or IgG_2 positive cells are detected in lymph nodes of colostrum-fed calves. The number of IgA and IgM positive cells, however, are similar in all calves (Aldridge et al., 1998). The most abundant immunoglobulin bearing cells in lymph nodes of neonatal calves are IgM positive cells (Aldridge et al., 1998), and there is a rapid increase in serum IgM concentrations of foals during the first month of life (Lavoie et al., 1989).

Colostrum-fed foals and calves are unresponsive to vaccines containing antigens to which high concentrations of maternal antibodies have been acquired (Husband and Lascelles, 1975; Van Maanen et al., 1992; Wilson et al., 2001; Munoz-Zanzi et al., 2002). However, age is also a factor, as colostrum-deprived calves are less responsive to vaccination at birth compared to calves vaccinated at four months of age. Colostrum-fed calves vaccinated with a novel protein (egg albumin) are able to mount a similar antibody response to colostrum-deprived calves (Husband and Lascelles, 1975).

Leukocytes and Complement Activity

Leukocyte antibacterial activity is not different between healthy human neonates and adults (Wright et al., 1975). Leukocyte antibacterial activity and polymorphonuclear lymphocyte (PMNL) chemotaxis in neonates is not affected by gestation length, birth weight, use of steroids, type of delivery (C-section, vaginal, local or general anesthesia), Apgar score, or neonatal therapy (antibiotics, calcium, assisted ventilation) (Wright et al., 1975; Krause et al., 1986). However, leukocytes from stressed neonates have reduced antibacterial activity compared to healthy neonates or adults (Wright et al., 1975). Also, PMNL chemotactic response is decreased in stressed neonates compared to healthy neonates or adults, but PMNL chemotaxis increases in stressed neonates during recovery (Krause et al., 1986). In contrast, PMNL adherence values are not different between healthy and stressed neonates; however, both values are decreased compared to adults (Krause et al., 1986).

Newborn calves (prior to colostrum ingestion) have higher total leukocyte counts than three to nine week old calves or adult cattle. The percentage of PMNL is higher, but percentages of monocytes and lymphocytes are lower (Zwahlen and Roth, 1990; Menge et al., 1998). Leukocyte counts do not change significantly after colostrum ingestion (Menge et al., 1998). However, total leukocytes, total lymphocytes and all lymphocyte subsets measured increase with age from birth through 120 d in foals (Smith et al., 2002).

Some aspects of neutrophil function are altered in the neonatal calf, lamb, and foal. Foal serum has lower opsonic capacity for neutrophils compared to adult serum through 14 d of age. Opsonic activity for yeast increases with age, and by three to four weeks, foal serum produces similar neutrophil phagocytosis compared to adult serum (Grondahl et al., 1999; Demmers et al., 2001). LeBlanc and Pritchard (1988) reported foal serum containing < 3.5

g/L of IgG has lower opsonizing activity compared to adult serum, whereas foal serum with > 6 g/L of IgG is similar in opsonizing activity compared to adults. Opsonic capacity of foal serum is greatly increased by colostrum ingestion, and postcolostral serum is able to opsonize *E. coli* to the same extent as adult serum (Grondahl et al., 2001). Lamb serum also provides decreased phagocytosis, with opsonic activity increasing after colostrum ingestion (Bernadina et al., 1991). Foal and lamb neutrophils have increased phagocytic ability when adult serum or IgG is used for opsonization, demonstrating that neonatal serum is the reducing factor for neutrophil phagocytosis in young animals (Bernadina et al., 1991; Grondahl et al., 1999).

Neonatal bovine neutrophils show enhanced migration compared to adult neutrophils (Zwahlen and Roth, 1990). Polymorphonuclear lymphocytes in newborn calves are unable to recognize and phagocytize bacteria as quickly as three to nine week old calves; however, monocytes have increased phagocytic abilities compared to three to nine week old calves. Ingestion of colostrum appears to decrease the time needed by phagocytic cells to recognize and ingest bacteria (Menge et al., 1998).

Neutrophil phagocytotic index increases and killing percent decreases from birth until approximately 113 d of age in foals, at which time these trends reverse. However, both indices were within the range for normal adult horses at all time periods (Wichtel et al., 1991). Foal neutrophils prior to suckling and at 28 d produce less chemiluminescence (CL) than adult neutrophils; however, at 14 d foal neutrophils produce more CL than adult neutrophils (LeBlanc and Pritchard, 1988). In contrast, Demmers et al. (2001) reported significantly lower neutrophil CL in foals at 2 d and 14 d of age compared to mature cells. In

this same study, neutrophil CL in foals that were three months of age or older was equal to or higher than mature horses.

All components of both the classical and alternative complement pathways are present in the newborn calf as evidenced by ability to hemolyze red blood cells. However, complement activity in both the alternate and classical pathway is decreased in newborn calves compared to their dams (Renshaw and Everson, 1979). Concentration of complement component C3 in calves at birth is approximately 28% of values observed in adult cows and is not changed by feeding colostrum. Concentrations increase significantly during the first month; however, they are still less than half of values observed in adult cows (Mueller et al., 1983).

Effects of Colostrum in the Neonate

First lactation cows produce significantly lower volumes of colostrum and lower amounts of colostral IgG compared to second or later lactation cows. More specifically, first lactation cows provide colostrum yields of 3.32 kg, containing 167 g (49 mg/ml) IgG, cows in their second to fourth lactation yield 8.10 kg containing 448 g (65 mg/ml) IgG, and cows in their fifth lactation or greater yield 6.74 kg and 445 g (86 mg/ml) IgG (Levieux and Ollier, 1999). However, others have reported that colostral IgG content does not differ between first and third lactation cows (Oyeniyi and Hunter, 1978; Pritchett et al., 1991). The weight of first colostrum is also negatively correlated with IgG concentration (Pritchett et al., 1991).

Besides high concentrations of IgG, first milking colostrum is higher in gross energy, crude protein, crude fat, γ-glutamyltransferase, aspartate aminotransferase, lactate dehydrogenase, and glutamate dehydrogenase compared to milk replacer (Hammon and Blum, 1998; Kuhne et al., 2000; Hammon and Blum, 2002). First milking colostrum also

contains approximately 20-25% more IGF-I, IGF-II, and insulin than mature milk (Odle et al., 1996; Kuhne et al., 2000; Hammon and Blum, 2002).

Colostrum-deprived calves have lower serum concentrations of γ-glutamyltransferase, aspartase aminotransferase, lactate dehydrogenase, and glutamate dehydrogenase after feeding compared to colostrum-fed calves; this change most likely reflects the high levels of these enzymes in colostrum (Kurz and Willett, 1991; Hammon and Blum, 1998; Rauprich et al., 2000). Kurz and Willett (1991) classified changes in lactate dehydrogenase as physiological changes relating to age, rather than relating to colostrum consumption.

Concentrations of growth hormone (GH) and IGF-1 are not different between calves fed colostrum or milk replacer (MR) during the first day of life. However, on day 2, calves that receive MR have lower IGF-1 concentrations compared to calves fed colostrum (Hadorn et al., 1997; Kuhne, et al., 2000). Insulin-like growth factor-1 concentrations in calves fed MR also decrease over the first 2 d of life, whereas calves fed colostrum maintain constant IGF-1 concentrations (Kuhne et al., 2000). In contrast, Rauprich et al. (2000) found plasma IGF-1 concentrations decreased over the first three days after birth in both colostrum and MR-fed calves.

Calves fed colostrum during day 1 have higher plasma phospholipid, cholesterol, carotene, and α-tocopherol concentrations at day 2, 3, 4, and 7, and plasma retinol concentrations are higher on day 2 and 3 compared to calves fed water or a glucose solution on day 1. Fat soluble vitamin, but not water soluble vitamin concentrations are also altered for the first 7 d of life in calves fed colostrum compared to colostrum-deprived calves (Blum et al., 1997).

Colostral leukocytes are also absorbed intact and contribute to neonatal immunity. Colostral leukocytes migrate intercellularly between duodenal and jejunal epithelial cells, but not ileal cells (Williams, 1993). Leukocytes are absorbed in greater quantities through jejunal Peyer's patches than through ileal patches and are observed passing to Peyer's patches through the follicle-associated epithelium (FAE). No labeled cells are detected in the epithelium outside the FAE (Liebler-Tenorio et al., 2002). In contrast, peripheral blood mononuclear cells harvested from the sow and fed to piglets are not absorbed into the circulation from the intestine (Williams, 1993).

Colostral leukocytes absorbed by neonates are able to exert immunomodulating effects. Piglets fed colostral leukocytes have higher T-cell responses and significant stimulation of B-cells compared to piglets not receiving colostral leukocytes (Williams, 1993). Calves infected with *E. coli* and receiving colostrum supplemented with colostral leukocytes shed fewer bacteria after infection than those calves receiving colostrum devoid of colostral leukocytes. Leukocyte supplemented calves also have higher total antibodies against *E. coli* by 2 d of age than calves fed colostrum devoid of colostral leukocytes (Riedel-Caspari, 1993).

Colostrum can have profound effects on development of the neonatal intestine. Villus circumference, area, and height and height/crypt depth ratio in the duodenum are greater in calves fed colostrum for at least the first three days after birth compared to colostrum-deprived calves or calves fed colostrum for 3 d or less (Buhler et al., 1998; Blattler et al., 2001). Blatter et al. (1998) observed no differences in the jejunum or ileum, whereas Buhler et al. (2001) observed differences throughout the intestine.

Cell turnover in the intestine is affected by feeding colostrum. Calves that are fed colostrum for the first 7 d of life have lower cell proliferation in the duodenum and ileum

compared to calves fed colostrum for 3 d. Colostrum-deprived calves also have lower cell proliferation in the duodenum compared to calves fed colostrum for 3 d (Blattler et al., 2001).

Total small intestinal villous circumferences, areas, and heights, are lower in calves injected with recombinant bovine GH compared to calves either fed or injected with IGF or fed colostrum alone. Thus, postnatal gut development in colostrum fed calves is not enhanced by administration of IGF or GH (Buhler et al., 1998). However, Rauprich et al. (2000) reported increased xylose uptake in calves fed colostrum compared to calves fed formula that contained only 15% of the IGF-I content of colostrum.

Binding sites for IGF-I and IGF-II are present in the intestinal mucosa of neonatal calves, with the greatest concentration of IGF-I sites in the distal intestine (ileum and colon) and IGF-II in the duodenum and colon. The number of binding sites for IGF-II is approximately 17 times greater than IGF-I in the newborn calf intestine, and decreases to five-times greater than IGF-I in 8 d old calves (Hammon and Blum, 2002; Georgiev et al., 2003). After birth, data for IGF-I and IGF-II in intestinal tissue is conflicting. Calves fed colostrum have a reduction in IGF-II binding sites in the ileum of the intestine at 7 d of age compared to calves fed MR with added globulins or MR with added rhIGF-I, suggesting that colostrum down-regulates IGF-II receptors in intestinal tissue (Baumrucker et al., 1994). In contrast, Hammon and Blum (2002) found an increase in numbers of IGF-II binding sites for calves fed colostrum for 3 d compared to colostrum-deprived calves. The number of IGF-I binding sites throughout the intestine was also higher in 8 d old calves fed colostrum for 3 d compared to calves fed colostrum at only one feeding and for colostrum-deprived calves (Hammon and Blum, 2002). Most recently, Georgiev et al. (2003) reported that IGF-I and IGF-II binding capacity was not affected by differences in nutrition in young calves.

Colostrum-deprived calves have lower dipeptidyl peptidase activity and higher maltase activity in the jejunum compared to calves fed colostrum for 3 d. Enzyme activity is not different between calves fed colostrum for 3 d and those fed colostrum for 7 d (Blattler et al., 2001). In contrast, pancreatic lipase activity and trypsin and elastase II activity are higher in calves fed colostrum for 7 d compared to calves fed colostrum for 3 d. There were no differences in pancreatic activity between calves fed colostrum for 3 d and colostrum-deprived calves (Blattler et al., 2001).

These differences between colostrum-fed and colostrum-deprived calves may ultimately affect gut absorptive ability. Calves fed colostrum have higher plasma xylose concentrations after administration of 0.5 g xylose/kg body weight than calves fed milk replacer, suggesting enhanced absorptive capabilities in colostrum fed animals (Hammon and Blum, 1997; Kuhne et al., 2000).

Failure of Passive Transfer (FPT)

Babies born at 32 weeks of gestation or earlier have circulating IgG concentrations below 4 g/L due to decreased placental transfer of IgG from the mother to the fetus (Hobbs and Davis, 1967). Babies with circulating IgG concentrations < 4 g/L have an increased incidence of severe infection compared to those infants with IgG concentrations > 4 g/L (Hobbs and Davis, 1967; Sandberg et al., 2000).

Colostrum is vital to the health and survival of neonatal calves and foals, and 18% of dairy cows provide colostrum with a total yield of less than 100 g of IgG, the commonly recommended amount needed to prevent failure of passive transfer in calves (Levieux and Ollier, 1999). Colostrum deprived calves are 50-75 times more likely to die before 21 d of age than colostrum fed calves, with most deaths occurring during the first week of life (Smith

and Little, 1922; Crowley et al., 1994; Wells et al., 1996). The risk of death for foals is also greatest during the first seven days after birth, with most deaths during this period attributable to septicemia (Cohen, 1994).

Calves, foals, and piglets with low IgG concentrations are at increased risk of morbidity and mortality (Boyd, 1972; McGuire et al., 1977; Frerking and Aeikens, 1978; Tyler et al., 1990; Clabough et al., 1991; Wittum and Perino, 1995; Rea et al., 1996; Donovan et al., 1998). The IgG threshold for FPT is questionable, and concentrations of < 5 g/L to < 12 g/L have been reported to be associated with increased disease and death (McGuire et al., 1977; Robison et al., 1988; Clabough et al., 1991; Wittum and Perino, 1995; Rea et al., 1996; Donovan et al., 1998). Virtala et al. (1999) reported that calves with serum IgG concentrations less than 12 g/L are two-times more likely to suffer from respiratory disease during the first three months of life than calves with IgG concentrations >12 g/L. A general classification for FPT is < 4 g/L for foals and < 8 g/L in calves (McGuire et al., 1977; Clabough et al., 1991; Wittum and Perino, 1995).

Thirty-one percent of foals have serum IgG concentrations < 8 g/L and 15-18% of foals have IgG concentrations < 4 g/L when sampled between 12-72 h (Clabough et al., 1991; Stoneham et al., 1991). Morris et al. (1985) reported the incidence of FPT was 2.9% in foals; however, this low value is explained by the fact that colostrum was provided by tube or bottle within 3 h of birth to foals in the study that either had trouble standing or nursing.

As stated earlier, calves and foals with low IgG concentrations are at increased risk for disease. All foals treated for neonatal infection, which could be attributable to colostral immunity, had serum IgG levels < 8 g/L (Stoneham et al., 1991). Colostrum-deprived calves have increased scour scores and decreased average daily gain compared to colostrum-fed

calves (Crowley et al., 1994). This increase in morbidity also results in lower weaning weights in calves with inadequate IgG concentrations at 24 h of age (Wittum and Perino, 1995).

Failure of passive transfer in foals is highly correlated to colostral specific gravity (FPT> with specific gravity <1.06) and mare age (FPT > when mare age is ≥ 15 yrs). Colostral specific gravity is highly correlated to IgG concentration, and mean colostral specific gravity and IgG concentration is greatest in mares 3-10 years old (LeBlanc et al., 1992). Failure of passive transfer in foals is also associated with the season foaled, type of delivery, gestation length, placental expulsion time, parity, and age of mare, listed in decreasing order of association (Clabough et al., 1991). Farms that assess passive immunity of foals also have lower morbidity; however, mortality rates are not different (Cohen, 1994). This is likely a management issue, as farms that assess passive immunity are also apt to aggressively manage ill animals.

Factors present in colostrum other than immunoglobulin molecules also aid in prevention of disease. Calves fed colostral Ig preparations containing IgG, IgA, or IgM alone experienced increased severity of disease in response to *E. coli* challenge compared to colostrum fed calves. However, colostral preparations were beneficial for reducing severity of disease when compared to calves fed colostral whey (Logan et al., 1974).

Exogenous Passive Immunity

Oral Colostral Supplements and Replacers

Oral colostral supplements (CS) and replacers (CR) are available and can be offered when either the dam's or other fresh or frozen colostrum source is not available. Colostrum supplements are preparations intended to provide < 100 g of IgG/dose and are not formulated

to replace colostrum. On the other hand, colostrum replacers contain > 100 g IgG/dose and provide additional nutrients required by the calf (Quigley et al., 2002b). Bovine colostrum can be used as a CS or CR for newborn foals and lambs. Foals fed 1-2 L of bovine colostrum attained peak serum IgG concentrations of 18-34 g/L (Holmes and Lunn, 1991), while lambs fed 50 g of bovine colostrum replacer had circulating bovine IgG concentrations of 66 g/L 24 after birth (Quigley et al., 2002a). However, bovine IgG, when fed to colostrum-deprived foals, has a serum half life of 7.4-9.6 days, which is much shorter than the half life for equine IgG (Lavoie et al., 1989; Holmes and Lunn, 1991). In contrast, bovine IgG supplied to lambs does not appear to be metabolized faster than ovine IgG (Quigley et al., 2002a).

Purified immunoglobulin can also be added to supplement poor quality colostrum, however, effects appear to be influenced by IgG source and mass of IgG provided. Morin et al. (1997) found no difference in serum IgG concentrations through 48 h of age in calves fed poor quality colostrum, or poor quality colostrum supplemented with either 136 g or 272 g of a dried colostrum supplement. Apparent efficiency of absorption for IgG is also lower (18% vs. 32%) for calves receiving poor quality colostrum supplemented with 272 g of a dried colostrum supplement compared to calves receiving poor colostrum only. Calves fed colostrum only received 102.8 g IgG1 in 12 h, while calves in the supplement group received 142.4 g and 185.2 g IgG1, respectively (Morin et al., 1997). Calves fed milk replacer supplemented with 50 g/L bovine IgG (25 g/L fed with 4 h after birth and again 4-6 h later) had serum IgG levels below 7 g/L throughout the 42 d experiment (Crowley et al., 1994). In contrast, medium and low quality maternal colostrum supplemented with bovine serum to provide equal total IgG concentrations to high quality colostrum alone have increased AEA at 24 h in calves (high quality-25%, medium quality-37%, low quality-38%). Calves

receiving medium and low quality supplemented colostrum also have higher serum IgG concentrations at 12 and 24 h of age compared to calves receiving high quality colostrum (Arthington et al., 2000b).

Calves fed 500 g of whey protein concentrate (WPC) either alone or in 1 L of pooled colostrum have lower serum IgG concentrations between 24-36 h and at three weeks of age compared to control calves (Mee et al., 1996). Also, mortality rate was higher and BW gain lower for calves receiving 500 g of WPC compared to calves receiving colostrum. However, there was no difference in mortality rate or BW gain for calves receiving 2 L of colostrum or 1 L of colostrum supplemented with 500 g WPC (Mee et al., 1996). The addition of whey protein concentrate or casein to colostrum supplement products does not appear to affect IgG absorption in neonatal calves unless the amount of total protein intake exceeds 500 g (Davenport et al., 2000).

Addition of immunoglobulin supplements to colostrum can also change absorption kinetics. Calves provided with colostrum that contains an additional 14 g of immunoglobulin supplement at birth and again 12 h later have alterations in IgG_1 concentration. Concentrations of IgG_1 in control calves peaks at 24 h of age and then decreases, whereas concentrations for supplemented calves does not change after peaking at 12 h of age (Abel-Francisco and Quigley, 1993).

Apparent efficiency of absorption of CS and CR varies widely based on product formulation. Apparent efficiency of absorption of IgG for calves fed maternal colostrum (MC) (200 g IgG), CS from bovine serum (90 g IgG), or two CS from milk-derived IgG (50 g and 60 g IgG) was higher for calves fed CS from bovine serum compared to MC or milk protein at 50 g of IgG (Arthington et al., 2000a). The AEA of IgG for bovine serum derived

products ranges from 20-33% (Quigley et al., 2000; Quigley et al., 2001; Quigley et al., 2002b). When equal masses of IgG are fed, serum IgG concentrations were higher in calves receiving bovine serum compared to colostrum or porcine serum (Arthington et al., 2000b).

Apparent efficiency of absorption of IgG in CR can also be affected by the amount of IgG fed. In comparison to MC, AEA is reduced when 750 g of CR is fed and increased when 266 g of CR is fed (Quigley et al., 1998). A CR derived from bovine Ig concentrate and providing 122 g of IgG resulted in plasma Ig levels at 24 h of 9.9-11 g/L when fed once immediately after birth and 12-14 g/L when fed twice; immediately after birth and at 8 h of age. In this same study, AEA was 29-32% when fed once and 19-20% when fed twice (Quigley et al., 2001). The addition of fat to CR does not affect IgG absorption (Quigley et al., 2001). Also, varying pH of colostral supplements between 7.5 and 5.0 does not influence AEA for IgG in calves (Quigley et al., 2000).

Calves administered three different commercial CS providing a total mass of IgG of 156, 107, and 126 g had lower IgG concentrations at 24 h and 48 h after birth, lower AEA, and more episodes of disease compared to calves fed maternal colostrum (total IgG mass of 164 g) (Garry et al., 1996). In contrast, other researchers have found that supplemental IgG can be provided to decrease severity of disease. Supplementation of milk replacer with bovine plasma powder decreased severity of clinical signs and increased survivability of calves infected with *E. coli* (Nollet et al., 1999). Calves infected with coronavirus and fed supplemental bovine serum were less dehydrated and maintain higher feed intakes compared to control calves (Arthington et al., 2002). Finally, calves provided with antibody against bovine coronavirus from egg yolk had lower mortality, lower fecal scores, and increased

weight gains after coronavirus infection compared to calves that did not receive antibody (Ikemori et al., 1997).

An oral equine serum product provided to foals within 4 h after birth (two doses 1-2 h apart) produced plasma IgG concentrations at 24 h of 1.05 g/L, which was well below values in control foals (39 g IgG/L) (Vivrette et al., 1998). However, foals allowed to suckle their dams and given two 150 ml doses of an equine serum product had higher serum IgG levels the day after treatment compared to foals that only suckle (19.4 g IgG/L treated vs. 12.4 g IgG/L control) (Davis et al., 1995).

Foals fed 250 ml lyophilized equine serum (5,400 mg of IgG/100ml) within 4 h after birth had similar serum IgG levels at 24 h of age compared to foals fed 250 ml of colostrum (7,200 mg of IgG/100ml) (Burton et al., 1981). Colostrum deprived foals given lyophilized purified equine IgG orally in 10 g units (diluted in 5% dextrose to 125 ml) at various times after birth have also been studied. Serum IgG concentrations of these foals did not differ whether they were given a total of 50 g, 60 g or 70 g of IgG. Concentrations of serum IgG were similar at 2 h, regardless of the initial amount of IgG given (range 10-30 g). Unfortunately, none of the dosing regimes provided serum IgG levels >5 g/L at 24 h of age (Franz et al., 1998). In general, a dose of 10 g of lyophilized IgG/15 kg body weight provided to foals after birth results in a mean serum IgG concentration of approximately 4 g/L at 14 h (Franz et al., 1998).

Injectable Immunoglobulin (calves and foals)

Once the period of intestinal permeability to immunoglobulin molecules has passed, passive immunity can be provided through intravenous (IV), intraperitoneal (IP), or subcutaneous (SQ) injection. Colostrum-deprived calves provided with 20 ml plasma/kg BW

intravenously have increases in total immunoglobulin of 262 mg %, in IgG_1 of 192 mg %, in IgG_2 of 66 mg %, in IgM 4 mg %, and in IgA of 0 mg% at 24 h. If given IP, total immunoglobulin increases 268 mg%, IgG_1 increases 192 mg %, IgG_2 increases 66 mg %, IgM increases 6 mg %, and IgA again increases 0 mg % at 24 h. However, values for circulating IgG concentrations were still below the protective level as evidenced by an increased prevalence of illness and lower average daily gain when compared to calves fed maternal colostrum (Anderson et al., 1987).

Subcutaneous injection of bovine plasma or purified plasma IgG containing 47 g or less of IgG does not raise plasma IgG levels in calves (Crowley et al., 1994; Quigley and Welborn, 1996). In contrast, calves provided with an IV infusion of a bovine plasma product containing 47 g IgG had increases in serum IgG of 2.9 g/L with an apparent infused IgG retention of 32%. However, mean BW gain, mortality and medical treatments were not affected by treatment in calves receiving IgG either by IV or SC injection (Quigley and Welborn, 1996).

Immunoglobulin G provided by intravenous infusion to calves and foals has a serum half-life of 18 - 23 d (Reilly and Macdougall, 1973: Besser et al., 1988). Approximately 1.5% is excreted daily in the feces, and 2.5% is excreted daily in the urine. It is estimated that 68% of the IgG_1 administered is transferred to the gastrointestinal tract (Besser et al., 1988).

Colostrum-deprived foals given an IV immunoglobulin (IVIG) concentrate containing 20 g or 30 g of equine IgG at a rate of 100 ml/10 min had serum IgG concentrations of 4.3 g/L and 7.7 g/L at 24-48 h post-infusion, respectively. The IgG concentration of the product was 10 g/100 ml, with foals receiving 200 ml and 300 ml, respectively (Liu et al., 1991). Brown et al. (1991) reported foals provided with IVIG containing 20 g, 30 g or 40 g of IgG had

average increases in serum IgG concentrations of 2.75 g/L, 3.40 g/L, and 4.84 g/L, respectively. Intravenous administration of 10 g lyophilized equine IgG diluted in 400 ml of 5% dextrose increased foal serum IgG concentrations by 2-3 g/L (Franz et al., 1998). Foals with variable initial IgG levels (1.95-6.58 g/L) had a mean increase in serum IgG levels of 4.82 g/L after receiving 3 L of IV equine plasma containing 12-16 g IgG/L (Brown et al., 1991). Serum IgG concentrations at 12 h post IV infusion are inversely related to foal body weight (Franz et al., 1998).

A minimum of 10 g of donor IgG should be administered IV to foals older than 1 d of age to raise serum IgG levels by 1 g/L (Stoneham et al., 1991). Pony foals administered equine plasma IV at a rate of 15 ml/kg after birth had serum IgG concentrations greater than 800 mg/dl at 24 h of age (Perkins et al., 2001).

Health status of the animal at the time of IVIG administration also affects outcome. Colostrum-deprived healthy foals provided IV equine plasma can be predicted to have an elevation in serum IgG of 8.68 mg/dl/g IgG administered/kg BW, where as clinically ill foals will have an elevation of 6.16 mg/dl/g IgG administered/kg BW, suggesting an increase in IgG metabolism or consumption by the ill foal (Wilkins and Dewan-Mix, 1994).

Pony foals provided with intravenous hyperimmune plasma containing *R. equi* antibodies had greater titers to *R. equi* 7 d after infusion compared to foals receiving normal plasma (Perkins et al., 2001). Surprisingly, mortality rate, duration of illness, and clinical parameters were not different between pony foals receiving hyperimmune *R. equi* or normal equine plasma that were infected with *R. equi* (Perkins et al., 2001).

Injectable Immunoglobulin (humans and lab animals)

Intravenous immunoglobulin is commonly used in human medicine to treat a variety of disorders and is reported to have a half-life of 24-30 d (Andresen et al., 2000). Intravenous immunoglobulin is purported to exert immunomodulatory properties in humans via the following mechanisms: 1) functional blockade of Fc receptors on splenic macrophages; 2) inhibition of complement damage by IgG binding of C3b and C4b fragments; 3) modulation of the production of cytokines; 4) neutralization of circulating autoantibodies; and 5) selection of immune repertoires (Andersson et al., 1996; Basta, 1996; Mouthon et al., 1996).

A study examining over 230 samples from commercially available human IVIG preparations detected no evidence of interleukin (IL)-6, IL-10, or tumor necrosis factor-α (TNF-α) in any of the preparations. However, high levels of transforming growth factor-β (TGF-β) were detected in all samples (Kekow et al., 1998). Neutralizing antibodies (specifically IgG) against the cytokines granulocyte-macrophage colony-stimulating factor, interfeuron-α (IFN-α), and IL-1 were also detected in over 70% of human immunoglobulin preparations tested. Neutralization of IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-12, TNF-α, and IFN-γ was not detected (Wadhwa et al., 2000).

Transforming growth factor-β concentrations increase in patients receiving IVIG; this is attributable to the high level of TGF-β contained in IVIG preparations (Kekow et al., 1998). However, the immunosuppressive effects of IVIG in vitro are not due to TGF-β, as inhibitory effects are not removed by addition of neutralizing antibody (Schaik et al., 1998).

Patients with primary hypogammaglobulinaemia have elevated levels of several components of the IL-1 system, IL-1 receptor antagonist, and of neutralizing IL-1α antibodies. Peripheral blood mononuclear cells (PBMC) from these patients also release

elevated levels of IL-1. Infusion of IVIG significantly decreased IL-1 levels in these patients, and PBMC also showed a decrease in the release of IL-1 (Aukrust et al., 1999). Serum obtained from IVIG patients after infusion also showed impaired IL-1α- and IL-1β-stimulated release of TNF-α from PBMC compared to that of serum collected prior to infusion (Aukrust et al., 1999).

Infusion of either IVIG or Fab_2 fragments inhibits TNF-α secretion from PBMC in vitro (Menezes, et al., 1997). In contrast, others have reported IVIG preparations inhibit in vitro PBMC proliferation and IL-4 secretion, but not IFN-γ or TNF-α (Andersson, et al., 1996; Campbell et al., 1999).

Human immunoglobulin, Fab_2, and Fc fragments all inhibit proliferation of PBMC in vitro; IgG and Fab2 fragments inhibit generation of cytotoxic T cells; and IgG and Fc fragments suppress natural killer cell lysis of target cells (Nachbaur et al., 1997). Immunomodulatory effects of human IVIG in vitro are attributable to IgG inhibition of T-cell proliferation and generation of cytotoxic T lymphocytes, and reduced IL-2 levels. Unchanged levels of IL-2 mRNA in IVIG supplemented cultures suggest that IVIG regulates IL-2 post-transcriptionally (Nachbaur et al., 1997).

Infants born after 32 weeks gestation or less have circulating IgG concentrations < 4 g/L have an increased incidence of severe infection compared to those infants with IgG concentrations > 4 g/L (Hobbs and Davis, 1967; Sandberg et al., 2000). Intravenous immunoglobulin is suggested as a method to increase disease protection; however, infants born with IgG levels < 4 g/L and receiving IVIG had no reduction in morbidity or mortality when compared to infants born with IgG levels < 4 g/L and given a placebo (Sandberg et al., 2000).

In contrast, immunoglobulin administered IP appears to increase survival in mice infected with type III Group B streptococcus (GBS), a common infectious agent in human infants. Mortality rates in newborn rats infected IP with GBS was 93% without treatment. Mortality rates were 0% when the rats were injected with hyperimmune human IgG IP at a dose of 140 mg IgG/kg immediately after GBS injection and at 30 min and 2 h post GSB injection. Mortality rates also decreased when IP IgG was given at 6 h and 12 h post GSB injection, but not 18 h post GSB injection (Givner and Nagaraj, 1993).

Immunoglobulin Products and Manufacturing

Plasma fractionation described by Cohn et al. (1946) is commonly used to isolate immunoglobulins. Using ethanol precipitation, Fraction II (FII) of this method contains the γ-globulins. Fractionation of human plasma using Gradiflow technology to purify IgG has also recently been described and recovers 95% of the IgG with high purity (Li et al., 2002).

Other methods of immunoglobulin separation have been described. Polyphosphate precipitation can be used to separate crude immunoglobulin from bovine plasma. Immobilized copper affinity chromatography or DEAE-Sephacel ion-exchange chromatography is then used to further purify the crude immunoglobulin. Immobilized copper affinity chromatography recovers approximately 40% of the IgG with a purity of 99%, while DEAE-Sephacel ion-exchange recovers 30% of the IgG with a purity of 100% (Lee et al., 1988).

Purified immunoglobulin for injection must be stabilized, and the pH and protein concentration must be adjusted prior to administration. An example is a liquid human IVIG prepared from plasma by Cohn FII, stabilized in sorbitol and with pH adjusted to 5.0. The protein concentration is then adjusted using 0.5 M HCl and 0.3 M glycine (Sisti et al., 2001).

Purified immunoglobulin can also be lyophilized. Lyophilized human IgG can be prepared from plasma by Cohn FII and stabilized with sucrose. Sodium concentration is adjusted to 120 mEq/L with 1.0 M NaCl, and pH adjusted to 7.0 using 10% $NaCHO_3$. The protein concentration is adjusted to 10% using 0.3 M glycine. The lyophilized product is reconstituted in sterile water prior to injection to provide a 5% IVIG solution (Sisti et al., 2001).

Polyethylene glycol (PEG) is a non-antigenic molecule that does not generate antibody production when coupled to other proteins (Abuchowski, 1977). When coupled to IgG, PEG stabilizes the IgG molecule, decreases complement fixing ability, and suppresses aggregate formation. Unfortunately, PEG-coupling also reduces antigen binding ability. PEG-coupled IgG with a PEG content of 4 mol/mol IgG retains about 70% of its antigen binding ability (Suzuki et al., 1984).

Polyethylene glycol can also be used to concentrate IgG from plasma (Polson and Ruiz-Bravo). However, calves fed CR containing bovine IgG concentrated with polyethylene glycol to fractionate the IgG from plasma had lower plasma IgG concentrations at 24 h and lower AEA compared to calves fed CR containing bovine IgG concentrate that was not prepared with polyethylene glycol fractionation (Quigley et al., 2001).

Water dilution, PEG, dextran sulfate, xanthan gum, and ammonium sulfate precipitation have all been described for purification of immunoglobulin (IgY) from egg yolk. Water dilution and ammonium sulfate precipitation both provide higher purity and yield when compared to the other methods (Akita and Nakai, 1993; Svendsen et al., 1995).

Reactions to Intravenous IgG infusions

Unfortunately, adverse reactions to IVIG administration can occur, and may be due to anaphylaxis, complement activation, vasoactive properties of the product, or other unknown mechanisms. Clinical signs of reactions vary in severity and include hyper- or hypotension, tachycardia, increased respiration rate, myalgia, rash, and nausea (Schifferli, 1992; Stangel et al., 1997). The clinical signs are usually associated with the release of preformed mediators from mast cells and basophils including the release of histamine (Bochner and Lichtenstein, 1991). Release of histamine is involved in anaphylactic-associated increase in vascular permeability, and adversely affects neutrophil infiltration (Hirasawa et al., 2002). Adverse reactions to IVIG in humans have also been shown to be associated with increases in IL-6 and thromboxane B_2, without concurrent changes in blood pressure, kininogen, histamine, or tryptase (Bagdasarian et al., 1998).

Incidence of adverse reactions to IVIG is often associated with rapid infusion rate (Stangel et al., 1997). With infusion rates of 0.04, 0.06, and 0.08 ml/kg/min for IVIG in humans (final dose of 0.5 g IgG/kg body weight), adverse reactions were only noted with the highest infusion rate (Bagdasarian et al., 1998).

Hypotension has been noted in rats administered a human IVIG product, with ranges from less than 15% up to a 50% decrease in mean arterial blood pressure (Bleeker et al., 1997; Bleeker et al., 2000). The long-acting hypotension observed in rats occurred after 1 min, and did not return to normal within 1-3 h after administration (Bleeker et al., 1987). The hypotensive effect was product related and was significantly correlated with the IgG dimer content of the IVIG product (Bleeker et al., 2000). Human IVIG products with a high content of IgG dimers induce a strong hypotensive effect in rats, however, there is a minimal

decrease in complement. On the other hand, heat aggregated IgG has little effect on blood pressure but leads to a decrease in complement. Thus, hypotension and complement activation are independent (Bleeker et al., 1987; Bleeker et al., 1989).

Dimer formation in IVIG preparations can be influenced by a variety of factors. The IgG dimer percentage in IV plasma products increases as the number of donors to the plasma pool increases, and some products may contain up to 40% IgG dimers (Tankersley et al., 1988). Immunoglobulin G dimer percentage also increases over time during storage, and with decreasing pH, increasing temperature, and increasing ionic strength (Tankersley et al., 1988).

Human IVIG products that contain elevated amounts of prekallikrein activator (PKA) (Hageman factor fragments) and kallikrein cause increased vascular permeability when injected into guinea pigs (Alving et al., 1980). Human plasma protein fractions with increased PKA concentrations also cause increased generation of bradykinin and decreases in mean arterial pressure when infused into rats (Bleeker et al., 1982). However, PKA, infused in concentrations equivalent to those found in standard human immunoglobulin preparations that caused hypotension in rats, had no effect on blood pressure in rats. A similar lack of hypotension was observed after infusion of a lower molecular weight fraction containing monomer IgG and 5% dimeric IgG. In contrast, the higher molecular weight fraction (containing IgG polymers) caused a strong hypotensive effect. Thus, it can be concluded that hypotension in response to IVIG administration was due to IgG aggregates in the preparation (Bleeker et al., 1987).

Immunoglobulin G aggregates prepared from human plasma bind and activate C1 (complement) in a dose-dependent fashion. Aggregates of 20 IgG molecules bind and

activate more C1 molecules/aggregate than IgG monomers. This data showed that monomeric IgG binds C1 poorly, whereas aggregation promotes binding. Aggregates as small as 5 IgG molecules greatly enhanced binding and activation over monomers of IgG (Doekes et al., 1982).

Incubation of human granulocytes with IVIG results in a concentration dependent increase in superoxide anion release that is mediated through the FcγR. Granulocytes exposed to IgG dimers release more O_2^- than those exposed to IgG monomers (Nemes et al., 2000).

Reactions to IVIG have also been observed in calves and foals. Calves receiving IVIG containing 47 g IgG that was purified and precipitated with $(NH_4)_2SO_4$ and suspended in 0.9% NaCl commonly demonstrated signs of transfusion reactions during infusion (Quigley and Welborn, 1996). Twenty-five to fifty percent of foals that received IVIG containing 20-30 g IgG showed mild signs of reaction including increased respiration rate, muscle tremors, tachycardia, hyperemic mucous membranes, depression, and mild signs of colic (Brown et al., 1991; Liu et al., 1991; McClure et al., 2001). An increased reaction rate is reported for foals receiving lyophilized IgG products as well. Fifty-five percent of foals receiving 10 g of lyophilized equine IgG showed signs of discomfort during infusion including diarrhea, tachypnea, and/or abdominal pain (Franz et al., 1998).

Reactions to Contrast Media/Hyperosmolar solutions

Administration of contrast media and hyperosmolar solutions to humans also causes reactions similar to those observed from infusion of IVIG. Table 1.2 lists many of the solutions/drugs that induce these reactions and the immune mechanisms activated.

Most general anesthetics and radiocontrast media induce the release of preformed mediators (histamine and typtase) from basophils and mast cells, not synthesized eicosanoids (PGD_2, LTC_4, and PAF) (Genovese et al., 1996). Increasing the osmolality of contrast media agents, as well as mannitol, causes an increase in histamine release from basophils (Findlay et al., 1981; Stellato et al., 1996). However, different amounts of histamine are released from basophils by contrast media in the same osmolality range suggesting that osmolality alone is not the sole mechanism influencing histamine release (Stellato et al., 1996). Histamine released by basophils in response to hyperosmolar mannitol is not a result of disruption of the cell membrane or cytotoxicity, and is independent from binding of IgE (Findlay et al., 1981; Findlay et al., 1984).

Table 1.2. Drugs and radiocontrast media frequently associated with anaphylactic/anaphylactoid reactions (Genovese et al., 1996).

Drug/RCM	IgE-Mediated Activation	Complement-Mediated Activation	Direct Activation
Muscle Relaxants (tubocurarine, suxamethonium, atracurium, pancuronium, vecuronium)	+	-	+
Hypnotics – barbiturates (Thiopental, methohexitone)	+	+	+
Nonbarbiturate hypnotics (Propofol, althesin, propanidid)	-	+	+
Opioids (Morphine, buprenorphine, fentanyl)	+/-	-	+
Plasma expanders (Dextran, hemagel)	-	+	+
Protamine	+	+	+
Radiocontrast media	+/-	+	+

Anaphylactic Reactions and Prevention

There are three ways that exposure to a substance can cause anaphylaxis: 1) exposure to a foreign protein that results in IgE antibody formation; reexposure results in IgE mediated degranulation of mast cells and basophils; 2) formation of immune complexes that activate the complement cascade; and 3) administration of certain agents (hyperosmolar solutions, radiocontrast agents, etc.) that directly stimulate release of mediators by unknown mechanisms (Bochner and Lichtenstein, 1991). Classic anaphylactic reactions are defined as resulting from a Type I immune response, also called immediate hypersensitivity. This type of reaction requires three components: 1) an antigen; 2) IgE antibody; and 3) effector cells such as mast cells and basophils that synthesize and release pharmacologic mediators (Carlson et al., 1986). Reactions that appear clinically similar to anaphylactic reactions, but are not mediated by IgE are referred to as anaphylactoid reactions (Carlson et al., 1986).

Mast cells are not necessarily required for anaphylactic shock in the mouse, as mast cell deficient mice are able to develop anaphylaxis clinically indistinguishable from that exhibited by normal mice (Jacoby et al., 1984; Arimura et al., 1990). Some studies report increased concentrations of blood histamine after challenge in control mice (Choi et al., 1998), while others report no change in histamine levels before or after anaphylaxis in both control and mast cell deficient mice (Jacoby et al., 1984). Hypotension and death due to anaphylactic reactions in mice also do not appear to require mast cell-derived mediators (Martin et al., 1993; Miyajima et al., 1997).

Systemic responses and mortality associated with anaphylaxis are mediated through FcRγ; mice lacking the FcRγ show no cardiopulmonary changes or mast cell degranulation, and do not die after induction of anaphylaxis. A functional FcεRI is also not required for

mast cell degranulation or mortality associated with anaphylaxis, as mice lacking this receptor exhibit responses similar to FcεRI +/+ mice (Miyajima et al., 1997).

In the mast cell deficient mouse, IgG_1 sensitizes other cells, resulting in release of platelet activating factor (PAF) and leading to the clinical signs of anaphylaxis (Arimura et al., 1990). Rat peritoneal cells produce PAF in response to either IgE, or IgG complexes. The production of PAF is unrelated to mast cell activation and depends on mononuclear cell activation via FcεRII or FcRγ (Pellon et al., 1993). The release of PAF results in hypotension, tachycardia, and circulatory collapse (Goldstein et al., 1991). Changes in mean arterial pressure are observed within one minute after intravenous injection of PAF (Rabinovici et al., 1991). Intravenous injection of PAF will also cause the death of mice in a dose dependent manner (Herbert et al., 1991; Fukuda et al., 2000), and topical application of PAF causes pronounced arteriolar vasoconstriction and leakage of FITC-dextran from the vascular space in the hamster cheek pouch (Klabunde and Anderson, 2000).

Incubation with IVIG causes activation of neutrophils and macrophages via Fc receptors resulting in PAF release (Bleeker et al., 1989; Teeling et al., 1998; Bleeker et al., 2000). Rabbits released increased amounts of PAF into their plasma within 2 min after antigen challenge during IgE-induced systemic anaphylaxis, and this release was indistinguishable both physiochemically and functionally from PAF released in vitro from IgE-sensitized basophils (Pinckard et al., 1979).

Anaphylactic shock in mast cell deficient mice was suppressed by CV-3988, a specific antagonist to PAF, but not by cyproheptadine, an antagonist to histamine and serotonin (Arimura et al., 1990). Pretreatment with PAF acetylhydrolase or PAF antagonist blocked fatal anaphylactic reactions in both mast cell deficient and control mice (Choi et al., 1998;

Fukuda et al., 2000). However, tachycardia in anaphylactic reactions is mediated by mast cells in mice, and cannot be prevented by antagonists to histamine, the cyclooxygenase metabolites of arachidonic acid, or PAF (Martin et al., 1993).

SK&F 98625 and SK&F 45905, inhibitors of coenzyme A-independent transacylase, caused concentration dependent reduction of PAF in stimulated neutrophils and mast cells (Winkler et al., 1996). Intravenous pretreatment with PAF acetylhydrolase completely protected mice from death induced by PAF injection (Fukuda et al., 2000). Pretreatment with SR 27417 (per os or intravenously) also protected mice from PAF-induced death (Herbert et al., 1991). Rupatadine is an orally active dual antagonist of histamine and platelet activating factor. This compound exhibited inhibitory effects for both PAF and histamine induced hypotension and bronchospasm in rats and guinea pigs (Merlos et al., 1997).

Conclusion

Passive immunity is important to neonatal animals for the prevention of disease, and there is a great body of literature supporting this fact. Management methods and products that significantly enhance passive immunity greatly enhance neonatal survival and decrease costs associated with morbidity. There are a variety of commercial products available that can be provided orally to calves and foals to increase passive immunity. However, the IgG content of many products is quite low, and most fail to increase IgG concentrations above levels needed to prevent FPT. Also, there are no commercially available concentrated IgG products for IV use in cattle, and minimal research on these types of products has been published.

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CHAPTER TWO

EFFECTS OF ORAL ADMINISTRATION OF CONCENTRATED EQUINE SERUM IGG TO NEWBORN FOALS ON PASSIVE IMMUNITY

A paper published in the Journal of Equine Veterinary Science[1]

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SUMMARY

Thirteen newborn foals of Quarter Horse breeding were used to determine if oral administration of concentrated equine serum increases concentrations of IgG in foals allowed to naturally suckle colostrum. Foals were alternately assigned either to receive 300 ml of an oral equine serum IgG product or to serve as controls. Foals receiving the IgG product were given 150 ml orally at 10 h and again at 12 h after birth. All foals were allowed to suckle from their dams *ad libitum*. Jugular blood samples were obtained from foals at 10 h and 24 h of age for IgG determination. Colostrum samples from the dam were also obtained within 3 h following parturition for determination of specific gravity. Plasma samples were analyzed for IgG level using a commercially available radial immunodiffusion kit. Oral administration of equine serum IgG had no significant effect on concentrations of plasma IgG in foals at 24 h of age ($p>.34$). There was also no difference between control and treated foals in the rate of IgG absorption from 10-24 h after birth ($p>.34$). In conclusion, oral administration of equine IgG to foals that ingest their dam's colostrum does not significantly increase concentrations of plasma IgG when compared to controls.

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INTRODUCTION

Colostral antibodies are the most important factor providing immunological protection to the neonatal foal.[1-3] Foals that fail to obtain adequate amounts of these antibodies, those classified with failure of passive transfer, are at an increased risk for developing infection and(or) death.[2,3] Foals with complete failure of passive transfer (IgG < 200 mg/dl) or partial failure of passive transfer (IgG 200-800 mg/dl) are often provided additional colostrum or colostral supplements when the foal is less than 24 h of age.[4] The oral colostral supplement tested in this study is commercially available and marketed to consumers not only as a treatment for failure of passive transfer, but also as a supplement to enhance antibody levels in foals with adequate passive immunity (Sera, Inc., Shawnee Mission, KS). The purpose of this study was to determine if an orally-administered concentrated IgG product can enhance IgG levels in normal foals that are allowed to suckle their dams *ad libitum*.

MATERIALS AND METHODS

Thirteen foals of Quarter Horse breeding were utilized in this study. All foals were born in individual stalls and parturition was attended from the onset of abdominal contractions. Foals were alternately assigned either to receive 300 ml of an oral equine IgG product of serum origin containing 12,000-15,000 mg IgG/300 ml (Sera, Inc., Shawnee Mission, KS) or to serve as a control. Approximately 10 ml of jugular blood was obtained from foals at 10 h after birth, centrifuged, and the plasma collected and stored at -20°C. Foals receiving oral IgG were given one dose immediately following the 10 h blood collection, and another dose at 12 h of age. Each dose of IgG was administered orally in three 50 cc aliquots using a dose syringe. A total of 150 ml was provided at each dosage time. Jugular blood samples were again obtained at 24 h of age from all foals, and plasma was collected and frozen. IgG

concentrations were determined by radial immunodiffusion using a commercially available kit (VMRD, Inc., Pullman, WA). The rate of IgG absorption (mg of IgG appearing in plasma/hr) was calculated to determine if oral supplementation affected this parameter.

All foals were allowed to suckle *ad libitum*. Colostrum samples were obtained from each mare within 3 h following parturition and specific gravity was determined using a colostrometer (Lane Manufacturing, Inc., Denver, CO).

All data were analyzed using the general linear models procedure of SAS.[5] Immunoglobulin G concentration at 10 h of age was included as a covariable for determining differences in concentration at 24 h of age, and rate of IgG absorption from 0-10 h of age was included as a covariable for determining differences in rate of absorption of IgG from 10-24 h of age. One foal in the treated group had difficulty suckling and had to be assisted during the first 12 h after birth; this foal was subsequently removed from the study.

RESULTS AND DISCUSSION

All foals had IgG concentrations well above 800 mg/dl at 10 h of age (Figure 2.1). In colostrum-fed foals, peak plasma IgG levels are obtained at approximately 12 h of age, so the decrease reported at 24 h of age for foals in the control group was expected.[1] The peak occurred closer to 18 h of age in supplemented foals, explaining the apparent increase in plasma IgG levels at 24 h of age in the treated foals in this study.

The specific gravity of colostrum produced by the mares was not different ($p>.53$) between the two treatment groups. Concentrations of IgG were higher for foals in the control group at 10 h of age ($p<.01$), and rate of IgG absorption (Figure 2.2) was increased from 0-10 h of age ($p<.01$) compared to that of treated foals.

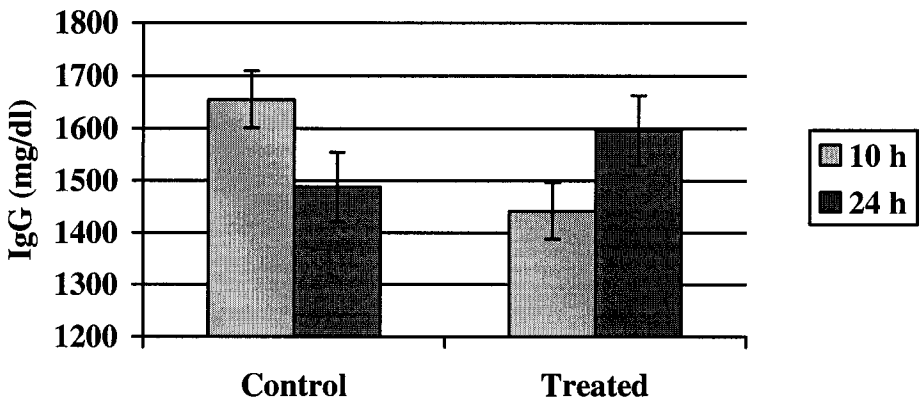

Figure 2.1. Concentrations of IgG prior to (10 h) and following (24 h) oral administration of concentrated equine serum derived IgG

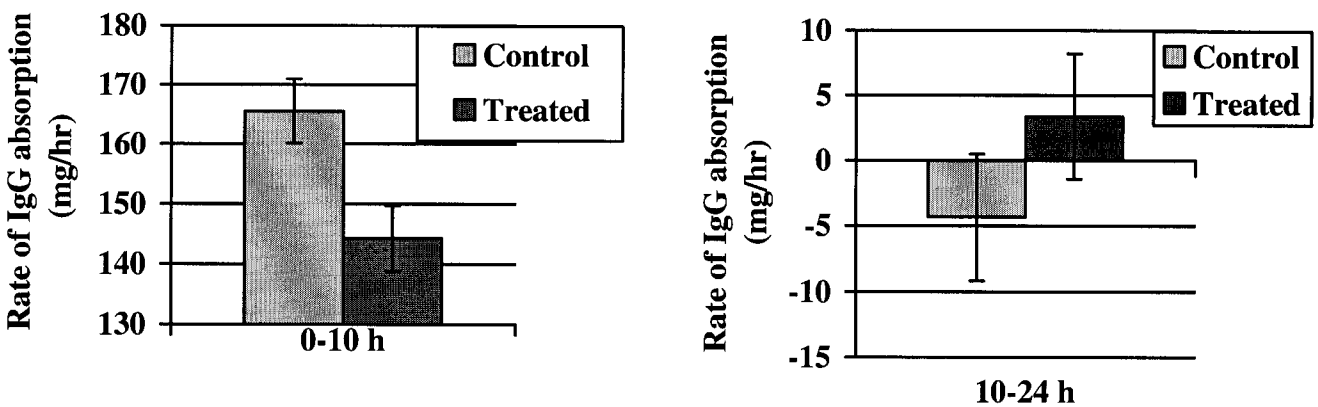

Figure 2.2. Rate of IgG absorption prior to (0-10 h) and following (10-24 h) oral administration of concentrated equine serum derived IgG

Although the foals were alternately assigned to treatments at birth, it is possible that by chance the more aggressive suckling foals or the foals with more maternal dams ended up in the control group. To account for these initial differences, foal plasma IgG concentration at 10 h and rate of IgG absorption from 0-10 h were included as covariables in the statistical analysis. When these differences were accounted for, plasma concentrations of IgG at 24 h of age and the rate of absorption of IgG from 10-24 h of age were not statistically different ($p>.34$) between foals in the two groups.

The results from this study indicate that this oral IgG product was ineffective in raising plasma IgG concentrations in foals allowed to suckle their dams *ad libitum* when administered at 10 h and 12 h after birth. A previous research study found that administration of this same product did in fact increase concentrations of IgG in foals at 24 h of age (treated mean [IgG]= 1935 mg/dl; untreated mean [IgG]= 1241 mg/dl).[6] The time of administration of the product was not reported, and so it is possible that earlier administration may have contributed to the different outcomes. However, other researchers have reported that oral administration of concentrated equine serum IgG within 4 h after birth does not raise concentrations of serum IgG above 500 mg/dl in colostrum deprived foals.[4,7] An increase in blood IgG concentrations of 200 - 300 mg/dl in foals with adequate passive immunity (IgG > 800 mg/dl) would provide minimal physiological improvement in disease resistance. If the colostrum supplement contained high enough titers of specific antibody not present in the dam's colostrum, then administration may be warranted. In conclusion, use of this product for increasing total immunoglobulin concentrations in normal foals suckling mares with good quality colostrum would not be beneficial.

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CHAPTER THREE

ADEQUACY OF A CONCENTRATED EQUINE SERUM PRODUCT IN PREVENTING FAILURE OF IMMUNE PASSIVE TRANSFER IN NEONATAL FOALS: A PRELIMINARY STUDY

A paper published in the Equine Veterinary Journal[1]

C.J. Hammer[2], J.A. Booth[3], L. Etzel[4], and H.D. Tyler[5]

Summary

The primary objective of this study was to determine if an orally administered concentrated equine serum product provided in the first hours of life could prevent failure of passive transfer in foals. To achieve this objective, ten foals of Quarter Horse breeding were utilized. Treated foals were administered 250 ml of an oral serum product at 1 and 3 h of age via nasogastric intubation. These foals were muzzled to prevent nursing from their dam. Supplemental milk replacer (200 ml/feeding) was provided to the treated foals at 6 h and 9 h of age. Mares of treated foals had their udder stripped at 1, 3, 6, and 9 h post parturition. The initial colostrum collected (200 ml) was fed back to the treated foals when the muzzle was removed at 12 h of age. Control foals were allowed to nurse from their dams ad libitum. Ten ml jugular blood samples were obtained from all foals (5 treated/5 control) at 1, 3, 5, 7, 9, 10, 11, 12, 24, and 48 h of age for determination of concentrations of plasma IgG. Plasma IgG concentrations were higher ($p<.05$) for treated foals compared to control foals at 5 h and 48 h of age. Plasma IgG concentrations were not different ($p>.10$) at all other time periods measured. All treated foals had plasma IgG concentrations over 700 mg/dl by 10 h of age.

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Introduction

Foals are essentially agammaglobulinemic at birth, and ingestion of adequate amounts of colostrum is essential to provide the neonatal foal with passive immunity (Jeffcott, 1974). Failure to obtain adequate passive immunity occurs in 15% or more of Thoroughbred and Standardbred foals, and results in increases in morbidity and mortality (Clabough *et al.* 1992; McGuire *et al.* 1977; Stoneham *et al.* 1991).

In an attempt to increase immunoglobulin levels in foals suffering from partial or complete failure of passive transfer (FPT), many oral and intravenous equine immunoglobulin supplements have been developed. If a foal is more than 24 hours old, intravenous products are the only option for administration, and must contain at least 30 g of purified IgG to increase serum IgG concentrations above 700 mg/dl (Liu *et al.* 1991; Wilkins and Dewan-Mix, 1994). If a foal is less than 24 hour old and FPT is confirmed or suspected based on history, oral immunoglobulin products can be administered if no banked equine colostrum is available.

Bovine colostrum can be offered and results in serum concentrations of IgG over 1300 mg/dl. Unfortunately, the half-life of bovine IgG in the foal is much shorter (7.4 - 9.4 d for bovine compared to 26 d for equine IgG) (Lavoie *et al.* 1989; Holmes and Lunn, 1991). Also, the ability of the bovine IgG to detect and present antigen to the foal's immune system successfully has not been fully evaluated.

Most oral equine immunoglobulin products contain relatively low IgG levels and fail to increase serum IgG concentrations above 500 mg/dl (Franz *et al.* 1998; Vivrette *et al.* 1998). Therefore, the objective of this study was to evaluate the adequacy of a concentrated oral purified equine serum product containing 36 g of IgG/dose.

Materials and Methods

Animals and collection of samples

Ten foals of Quarter Horse breeding were alternately assigned to either the treated group or the control group. The dams of the foals ranged in age from 4 to 21 years. Treated foals were administered 250 ml of an oral serum product at 1 h and 3 h of age via nasogastric intubation. These foals were muzzled to prevent nursing from their dam. Supplemental milk replacer[1] (200 ml/feeding) was provided to the treated foals at 6 h and 9 h of age. Mares with treated foals had their udders stripped at 1, 3, 6, and 9 h post parturition. The initial colostrum collected (200 ml) was fed back to the treated foals when the muzzle was removed at 12 h of age. Control foals were allowed to nurse from their dams ad libitum. Ten ml jugular blood samples were collected into tubes using EDTA as the anticoagulant. Samples were obtained from all foals (5 treated/5 control) at 1, 3, 5, 7, 9, 10, 11, 12, 24, and 48 h of age for determination of concentrations of plasma IgG.

Oral serum product

Equine serum was purchased from a closed herd of horses[2] and the IgG was concentrated to 72% purity and verified by radial immunodiffusion[3]. The purification process involved enriching the IgG through standard chemical precipitation, which resulted in the removal of albumin. The spray dried product was balanced with 1% dextrose and 0.1M glycine and mixed in 250 ml of warm distilled water prior to feeding. Each dose contained 36 g of IgG for a total dose of 72 g (2 doses/treated foal x 36 g/dose).

Immunoglobulin quantification

Plasma was harvested from all blood samples by centrifugation and IgG concentrations were determined by radial immunodiffusion[3]. Personnel performing the IgG assay were blinded as to the grouping of the foals and the sample collection time.

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) procedure of SAS[4] and included foal within treatment interaction as the error term. Independent effects included in the statistical model were birth weight, dam parity, dam age, and foal sex. Initial IgG level at 1 h was included as a covariable. Significance was declared as P values less than 0.05.

Results

Average foal weight was 50 kg (S.D. ± 6 kg) for control foals and 47 kg (S.D. ± 5 kg) for treated foals. Mean values and ranges for plasma IgG concentrations at all sampling periods are presented in Table 3.1. Plasma IgG concentrations were higher ($p<.05$) for treated foals compared to control foals at 5 h and 48 h of age. Plasma IgG concentrations were not different ($p>.10$) at all other time periods measured. All treated foals had plasma IgG concentrations in excess of 700 mg/dl by 10 h of age.

Mean pre-suckling colostrum values at 1 h were 14,466 mg/dl for dams of control foals and 11,714 mg/dl for dams of treated foals. There was no difference in colostrum IgG concentration between the two groups of foals.

Discussion

There are few viable options for producers within the first 24 hours after a foal is born to prevent failure of passive transfer if supplemental colostrum is not available. The oral equine products currently marketed fail to raise IgG levels to an adequate level, however, there is

Table 3.1. Mean immunoglobulin G values (mg/dl) and ranges for control and treated foals

	Hours after Birth									
	1	**3**	**5**	**7**	**9**	**10**	**11**	**12**	**24**	**48**
Control	33.0	93.8	308.6*	552.0	697.6	760.2	829.6	815.2	747.8	661.2*
(±S.D)	(73.8)	(62.3)	(152.4)	(264.8)	(313.9)	(252.3)	(266.7)	(256.8)	(205.1)	(198.4)
Range	0-165	0-159	107-498	217-952	297-1141	464-1143	491-1225	554-1234	590-1099	460-955
Treated	22.8	139.4	534.0*	802.8	826.0	825.2	844.6	797.4	965.2	943.0*
(±S.D)	(50.9)	(50.1)	(81.8)	(170.1)	(178.3)	(141.5)	(153.8)	(153.7)	(205.5)	(174.8)
Range	0-114	81-215	457-664	644-1087	661-1029	707-1012	714-1024	597-995	717-1272	753-1213

* $P < 0.05$

some evidence that they can provide protection against illness (Franz *et al.* 1998). Intravenous IgG products not only raise foal serum immunoglobulin levels, but also have the advantage that they can be administered to a foal of any age. Drawbacks to intravenous IgG products include both the invasive nature of administration and the risk of adverse systemic reactions. Typical reactions include tachypnea, tachycardia, shaking, depression, diarrhea, abdominal discomfort and hyperemic mucous membranes (Liu *et al.* 1991; Madigan 1997). Therefore, oral products provide a safe alternative for foals from mares with a history of inferior colostrum quality, foals with a history suggestive of FPT, or simply as a preventative measure.

Two foals from the current study (one control/one treated) had measurable IgG concentrations at 1 h even though all births were attended and the foals did not nurse prior to the sample. This was not surprising as a small percentage of newborn calves and foals have been reported to have minimal IgG levels at birth in other studies (McGuire *et al.* 1973; Edwards *et al.* 1982). To account for this, IgG concentrations at 1 h were included in the statistical model.

Concentrations of IgG in the control foals were relatively low. It is possible that the frequency of sampling interrupted normal foal behavior and resulted in a decrease in suckling as one control foal still had an IgG concentration of zero at 3 h. If control foal IgG concentrations had been higher, there may have been significant differences between the two groups of foals, however, all treated foals still attained IgG levels above 700 mg/dl and two foals achieved levels greater than 1000 mg/dl.

Even though foals from this study were allowed to nurse their dam after 12 h of age, the results show that adequate plasma IgG levels can be achieved through the routine oral

administration of a concentrated immunoglobulin product within the first 5 hours after birth. The increased concentration of IgG observed in the treated foals can be attributed to the high concentration of IgG contained in the serum product administered in this study.

Plasma IgG values were higher at 48 h in treated foals compared to control foals; this can be explained by the administration of dam's colostrum after the 12 h sample. It is also possible that the equilibrium process was altered in the treated foals by the 12 h colostrum feeding. It takes approximately 2-3 d for IgG to reach equilibrium between the intravascular and extravascular space and this process may have been delayed in the treated foals by the 12 h colostrum administration (Reilly and MacDougall, 1973).

Because this is a newly developed product, further studies are needed to test the efficacy of the product for prevention of illness in colostrum deprived foals. The preliminary results from this trial support the use of this product for prevention of FPT in newborn foals.

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Manufacturer's Addresses

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[2] Nova-Tech, Grand Island, NE, USA

[3] Equine RID, Triple J Farms, Bellingham, WA, USA

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CHAPTER FOUR

CHARACTERIZATION OF A COLOSTRUM REPLACER CONTAINING IgG CONCENTRATE AND GROWTH FACTORS

A paper to be submitted to the Journal of Dairy Science

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ABSTRACT

The objective of this study was to characterize absorption of colostrum replacer (CR) or supplement (CS) containing fractions of bovine plasma. Immunoglobulin concentrate (IGC) was prepared from bovine abattoir blood to a final purity of approximately 90% IgG. Bovine blood was also processed to produce a fraction containing elevated concentrations of IGF-1 and TGF-β (GF). Both IGC and GF were spray-dried and blended with other ingredients to produce CR (30% IgG) or CS (15% IgG) containing 0 or 5% GF. Holstein bull calves (n = 40) were removed from the dams immediately after birth and assigned to one of five treatments: 1) Pooled colostrum (MC) - 1.9 L of pooled colostrum at 1 and 8 h of age; 2) Low supplement (LC) - 1.9 L of CS at 1 and 8 h of age to provide 150 g of IgG; 3) Low supplement + GF (LG) - 1.9 L of a CS with GF at 1 and 8 h of age to provide 150 g of IgG; 4) High supplement (HC) - 1.9 L of CR at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial milk replacer (MR) at 8 h of age; and 5) High supplement + GF (HG) - 1.9 L of a CR with GF at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial MR at 8 h of age. Blood was collected by jugular venipuncture at 0 and 24 h for determination of plasma IgG. Five calves fed HG, HC, and MC received an oral xylose solution (0.5 g/kg body weight) at 2 d of age. Jugular blood samples were obtained at 0 and 2 h after xylose

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ingestion. Apparent efficiency of IgG absorption (AEA) for IgG was higher (p=.02) for calves fed HC and HG compared to those fed LC and LG and was lower (p=.03) for calves fed LG and HG compared to those fed LC and HC. IgG concentrations at 24 h were highest (p<.0001) in calves fed MC compared to other calves and were higher (p=.048) in calves fed HC and HG compared to LC and LG. Calves fed LG and HG had lower (p=.02) IgG concentrations at 24 h of age compared to those fed LC and HC. Xylose absorption was not influenced by treatment. These results indicate that 150 g of IgG provided in one dose soon after birth is superior to 150 g of IgG fed in two doses 7 h apart. Also, addition of a fraction of bovine plasma containing elevated concentrations of IGF-1 and TGF-β to the CS and CR formulation decreased IgG absorption in the neonatal calf.

Abbreviation key: AEA = apparent efficiency of IgG absorption, **CR** = colostrum replacer, **CS** = colostrum supplement, **FPT** = failure of passive transfer, **GF** = serum fraction with elevated IGF-1 and TGF-β, **HC** = high supplement; **HG** = high supplement plus GF; **IGC** = immunoglobulin concentrate, **IGF-1** = insulin like growth factor –1, **LC** = low supplement; **LG** – low supplement plus GF; **MC** = pooled colostrum; **MR** = milk replacer; **TGF-β** = transforming growth factor β.

INTRODUCTION

Colostrum is vital to the health and survival of the neonatal calf, and 18% of dairy cows provide colostrum yields with less than 100 g of IgG, the most commonly recommended amount to prevent failure of passive transfer **(FPT)** (Levieux and Ollier, 1999). Colostrum-deprived calves are 50-75 times more likely to die before 21 d of age than colostrum-fed calves, with most deaths occurring during the first week of life (Smith and Little, 1922; Crowley et al., 1994; Wells et al., 1996). Therefore, colostrum supplements **(CS)** and

colostrum replacers (**CR**) have been developed that can be provided when colostrum is either of poor quality or unavailable. Colostrum supplements are preparations intended to provide < 100 g of IgG/dose and are not formulated to replace colostrum. On the other hand, colostrum replacers contain > 100 g IgG/dose and usually provide additional nutrients required by the calf (Quigley et al., 2002).

Colostrum not only provides passive immunity for the newborn calf, but it can also have profound effects on the development of the neonatal intestine. Villous circumference, area, and height and height/crypt depth ratio in the duodenum are higher for calves fed colostrum compared to colostrum-deprived calves (Buhler et al., 1998; Blattler et al., 2001). Calves fed colostrum also have higher plasma xylose concentrations after administration of xylose compared to calves fed milk replacer (**MR**), suggesting enhanced absorptive capabilities in colostrum fed animals (Hammon and Blum, 1997; Kuhne et al., 2000). Rauprich et al. (2000) also reported increased xylose uptake in calves fed colostrum compared to calves fed formula similar to colostrum but containing 15% of the insulin-like growth factor-1 (**IGF-1**) content. However, Buhler et al. (1998) found no enhancement of gut development in colostrum fed calves after oral or subcutaneous injection of IGF-1. Therefore the objective of this study was to characterize the absorption of colostrum replacer (CR) or supplement (CS) containing a fraction of bovine plasma with elevated concentrations of IGF-1 and transforming growth factor-β (**TGF-β**).

MATERIALS AND METHODS

Diet formulation

Immunoglobulin concentrate (**IGC**) was prepared from bovine abattoir blood to final purity of approximately 90% IgG. The blood was also processed to produce a fraction

containing elevated concentrations of IGF-1 and TGF-β (**GF**). Both IGC and GF were spray-dried and blended with other ingredients to produce CR (30% IgG) or CS (15% IgG) and either containing 0 or 5% GF. Samples of each diet were analyzed for IgG content (Etzel et al., 1997) and for proximate nutrients according to AOAC procedures at a commercial facility (Silliker Inc., Minnetonka, MN).

Experimental procedure

Holstein bull calves (n = 40) were removed from their dams immediately after birth and assigned to one of five treatments: 1) Pooled colostrum **(MC)** - 1.9 L of pooled colostrum at 1 and 8 h of age; 2) Low supplement **(LC)** - 1.9 L of CS at 1 and 8 h of age to provide 150 g of IgG; 3) Low supplement + GF **(LG)** - 1.9 L of a CS with GF at 1 and 8 h of age to provide 150 g of IgG; 4) High supplement **(HC)** - 1.9 L of CR at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial milk replacer (MR) at 8 h of age; and 5) High supplement + GF **(HG)** - 1.9 L of a CR with GF at 1 h of age to provide 150 g of IgG and 1.9 L of a commercial MR at 8 h of age. Experimental diets were reconstituted in water and mixed in a household blender until well blended. The mixture was then poured into a nipple bottle and offered to the calf. Amounts not voluntarily consumed were provided via an esophageal feeder.

At 0 and 24 h of age, blood was collected from all calves by jugular venipuncture into evacuated tubes containing EDTA. A sample was placed in a microhematocrit tube and centrifuged for hematocrit determination. Plasma was collected by centrifugation and total protein was determined with a handheld refractometer (Schuco Clinical Refractometer). Remaining plasma was frozen (-20°C) for later determination of IgG by turbidimetric immunoassay (Etzel et al., 1997).

Xylose absorption

Five calves each from the treatment groups HC, HG, and MC were administered an oral xylose solution (0.5 g *d*-xylose/kg body weight) at 2 d of age approximately 4 h after the morning meal. Blood samples were obtained from each calf via jugular venipuncture prior to xylose administration and again at 2 h after xylose ingestion. Plasma was collected by centrifugation and frozen (-20°C) for later spectrophotometric analysis (Merritt and Duelly, 1983).

Statistical analysis

Experimental data were analyzed using the general linear models procedure of SAS (1996). Orthogonal contrasts were used to test differences between MC and CR, low IgG dose and high IgG dose, and GF addition. Chi square analysis was used to determine differences in FPT. Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

The composition of the experimental CS and CR, as well as the MR provided to calves on treatment HC and HG, is provided in Table 4.1. The CP% was slightly higher for HC and HG diets, however, total protein consumed was similar between treatments since calves on treatment HC and HG were fed MR at 8 h of age. The composition of GF is provided in Table 4.2. Four calves did not survive until the 24 h blood sample, and were therefore excluded from the analysis. Mean BW of calves did not differ among treatments (Table 4.3). The frequency of feeding via esophageal feeder also was not different among treatments.

Table 4.1. Composition of experimental colostrum supplements, colostrum replacers, and milk replacer.

Item[1], %	LC	LG	HC	HG	MR
DM	96.8	93.6	95.5	93.6	97.1
CP	31.3	32.1	40.3	41.5	21.3
Ash	5.7	5.5	4.8	4.8	7.1
Fat	19.8	23.6	23.1	23.8	23.6

[1]Items are on a DM basis, except for DM
[2]MR = commercial milk replacer provided to calves in treatments HC and HG at 8 h of age

Table 4.2. Composition of serum fraction GF.

Item[1]	Value
DM, %	96.6
TP, %	93.24
Albumin, %	56.64
IgG, %	19.7
Ash, %	0.61
TGF-β_1, ng/g	765
TGF-β_2, ng/g	9
IGF-1, ng/g	1368
Standard plate count, CFU/g	600
Endotoxin, EU/g	0.311

[1]Items are on a DM basis, except for DM

Total IgG intake was 282 g for calves fed MC and 150 g for calves fed all other treatments. The GF fraction contributed an additional 4 g of IgG to calves receiving those diets. Immunoglobulin G concentrations (Table 4.3) were highest at 24 h of age in calves fed MC compared to calves in the other four treatment groups, which is easily attributable to the difference in IgG intake. Immunoglobulin G concentrations were higher at 24 h of age in calves fed HC and HG compared to LC and LG, even though the total IgG intake was equal between all four groups. Apparent efficiency of IgG absorption **(AEA)** (Table 4.3) was also higher for calves fed HC and HG compared to those fed LC and LG. These results contradict previous findings that show no difference in serum IgG concentrations and similar AEA after feeding calves a large amount at birth or the same amount divided into two or three feedings

(Hopkins and Quigley et al., 1997; Morin et al., 1997). However, the observed differences may be related to the fact that comparisons in the previous studies were based on feeding maternal colostrum, whereas calves in the current study received CS or CR. The AEA for colostrum replacers derived from bovine Ig concentrate has been shown to be higher when fed once compared to feeding twice after birth 8 h apart (Quigley et al., 2001). The values for AEA observed in this trial are similar to those previously reported for CS and CR prepared from bovine plasma (Quigley et al., 2002). Immunoglobulin G concentrations were higher in calves fed the CS and CR formulations than those previously reported by Quigley et al. (1998) for calves receiving 150 g IgG obtained from bovine serum.

Calves fed LG and HG also had lower IgG concentrations at 24 h of age and lower AEA compared to those fed LC and HC, suggesting that addition of GF to the CS and CR formulation negatively influenced IgG absorption. Concentrations of TGF-β and IGF-1, although concentrated in the serum fraction, were still well below the concentrations found in bovine colostrum. Values for IGF-1 and TGF-β1 in cow colostrum range from 203-1850 ng/ml and 12.4-42.6 ng/ml, respectively (Hadorn et al., 1997; Ginjala and Pakkanen, 1998). The fraction of bovine serum may have contained other compounds that either negatively affected intestinal absorption or somehow increased IgG metabolism, although at this time it is unknown what those compounds may be.

Preadministration xylose concentrations (Table 4.4) were slightly higher than those previously reported. Others have reported preadministration xylose concentrations ranging from 7-13 mg/dl in young calves fed colostrum, whole milk, or milk replacer (Seegraber and Morrill, 1979; Kuhne et al., 2000). Hammon and Blum (1997) reported even lower concentrations of 4-5 mg/dl in calves. Post-administration xylose samples were obtained 2 h

after xylose administration and were not different among treatments. Peak xylose values are obtained 2-2.5 h after administration of xylose in fasted calves (Seegraber and Morrill, 1979); however, peak values are reached later in calves fed xylose with the morning meal (Kuhne et al., 2000; Rauprich et al., 2000). Previously reported peak xylose values average from 42-56 mg/dl over the first 5 weeks of life, and are similar for fasted and non-fasted calves (Seegraber and Morrill, 1979; Kuhne et al., 2000). Although the post-administration xylose concentrations were higher in this study, the change in xylose concentration was similar to previously reported values (Seegraber and Morrill, 1979).

Table 4.3. Least squares means of treatment parameters for calves fed LC, LG, HC, HG and MC.

	Treatment[1]						Contrasts[2]		
Item	MC	LC	LG	HC	HG	SE	1	2	3
No. calves	7	7	7	8	7	...	...	...	...
BW, kg	49.37	47.30	45.35	45.18	49.24	1.79	NS[3]	NS	NS
IgG intake, g	282.2	150	150	150	150	9.4	0.001	NS	NS
Plasma IgG, g/L									
0 h	0	0	0	0	0	0	NS	NS	NS
24 h	18.07	10.62	9.14	12.96	10.33	0.86	0.001	0.05	0.02
Change 0-24 h	18.07	10.62	9.14	12.96	10.33	0.86	0.001	0.05	0.02
AEA %	29	30	24	35	30	2	NS	0.02	0.03
FPT[4], %	0	42	57	0	28	14	0.06	0.02	NS
Plasma protein, g/L									
0 h	4.75	4.62	4.37	4.57	4.51	0.15	NS	NS	NS
24 h	6.12	5.02	5.20	5.24	4.91	0.17	0.001	NS	NS
Change 0-24 h	1.37	0.40	0.86	0.61	0.40	0.15	0.001	NS	NS
Hematocrit, %									
0 h	40.14	42.57	33.14	37.62	39.28	2.62	NS	NS	0.09
24 h	34.14	35.85	26.66	33.85	36.71	1.87	NS	0.04	NS
Change 0-24 h	-6.0	-6.71	-5.50	-2.71	-2.57	1.82	NS	0.07	NS

[1]MC = 1.9 L of maternal colostrum at 1 and 8 h; LC = 1.9 L of CS at 1 and 8 h; LG = 1.9 L of CS with GF at 1 and 8 h; HC = 1.9 L of CR at 1 h and 1.9 L of MR at 8 h; HG = 1.9 L of CR with GF at 1 h and MR at 8 h

[2]Contrasts: 1 = MC vs. CR; 2 = Low vs. High; 3 = 5% GF vs. 0% GF

[3]$P > 0.10$

[4]Failure of passive transfer (IgG < 10 g/L)

Table 4.4. Least squares means for xylose absorption in calves fed HG, HC, and MC.

	Treatment[1]				Contrasts[2]	
Item	MC	HC	HG	SE	1	2
No. calves	5	6	5	...	...	...
Xylose absorption, mg/dl						
0 h	16.56	15.22	15.89	0.56	NS[3]	NS
2 h	71.55	55.97	60.76	9.0	NS	NS
Change 0-2 h	54.98	40.74	44.87	8.9	NS	NS

[1]MC = 1.9 L of maternal colostrum at 1 and 8 h; HC = 1.9 L of CR at 1 h and 1.9 L of MR at 8 h; HG = 1.9 L of CR with GF at 1 h and MR at 8 h
[2]Contrasts: 1 = MC vs. CR; 2 = 5% GF vs. 0% GF
[3]$P > 0.10$

These results contradict those previously reported that show higher plasma xylose concentrations after administration of xylose in colostrum fed calves compared to calves fed MR (Hammon and Blum, 1997; Kuhne et al., 2000). However, calves in the current study were fed CR at one feeding and MR at the second, compared to the previous trials in which calves were fed MR only. Hammon and Blum (1997) observed no difference in xylose absorption when calves were fed maternal colostrum at only the first feeding or for more than one feeding. The lack of difference in xylose absorption after addition of IGF-1 and TGF-β also supports Buhler et al. (1998) who reported no enhancement of gut development after oral administration of IGF-1.

CONCLUSIONS

Newborn calves readily absorbed the IgG concentrate used in this study, and plasma IgG concentrations at 24 h of age were indicative of successful passive transfer. The results from this study indicate that 150 g of IgG provided in one dose soon after birth is superior to 150 g of IgG fed in two doses 7 h apart. These data also suggest that addition of a fraction of bovine plasma containing elevated concentrations of IGF-1 and TGF-β to the CS and CR formulation decreased IgG absorption in the neonatal calf. Further studies are needed to

determine how growth factors influence intestinal immunoglobulin absorption in the neonatal calf.

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CHAPTER FIVE

EFFECTS OF DIFFERENT LEVELS OF PASSIVE IMMUNITY ON RESPONSE TO INFUSION OF INTRAVENOUS IMMUNOGLOBULIN IN CALVES

A paper to be submitted to the Journal of Dairy Science

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ABSTRACT

The objective of this study was to determine the effect of different levels of passive immunity on calf response to administration of intravenous immunoglobulin (IVIG). The IVIG was concentrated from bovine abattoir blood to a final concentration of approximately 35 g IgG/L. Dairy breed bull calves (n = 32) were removed from their dams immediately after birth and assigned to one of four treatment groups. Calves in the high group (H) received 2 L of pooled MC at 1 h and 12 h after birth. Calves in the low group (L) received 1 L of MC mixed with 1 L of milk replacer at 1 h and 12 h after birth. Calves in the deprived group (D) received 2 L of milk replacer at 1 h and 12 h after birth. Calves in the control group (C) received 2 L of pooled MC at 1 h and 12 h after birth. At 3 d of age, calves in the H, L, and D group all received 500 ml of IVIG administered via jugular catheter. Calves in the control group received 500 ml of 0.9% NaCl. Blood was collected by jugular venipuncture prior to infusion, and again at 24 h post infusion for determination of plasma IgG by turbidimetric immunoassay. Mean plasma IgG concentrations at 3 d of age were different between the H, L, and D group of calves, but not between H and C. Mean plasma IgG at 3 d of age were 12.1, 6.3, 0.0, and 11.8 g/L for calves in H, L, D, and C, respectively.

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Calves in all treatment groups had a greater rise in mean plasma IgG compared to C calves at 24 h post infusion. Mean increase in plasma IgG at 24 h post infusion were 1.8, 2.4, 3.0, and –0.1 g/L for calves in H, L, D, and C, respectively. These data indicate that IVIG can increase plasma IgG levels in calves regardless of the level of passive immunity present at infusion.

Abbreviation key: FPT = failure of passive transfer, **IVIG** = intravenous concentrated plasma product, **MC** = maternal colostrum, **MR** = milk replacer.

INTRODUCTION

Calves are essentially agammaglobulinemic at birth, although a small percentage of calves may have negligible amounts of circulating serum IgG_1, IgG_2, IgM, and IgA. In ruminants, passive immunity is provided through ingestion of colostrum by the neonate after birth (Smith and Holm, 1948; Klaus et al., 1969). Colostrum is vital to the health and survival of the neonatal calf, and 18% of dairy cows provide colostrum yields with less than 100 g of IgG, the most commonly recommended amount to prevent failure of passive transfer **(FPT)** (Levieux and Ollier, 1999). Colostrum-deprived calves are 50-75 times more likely to die before 21 d of age than colostrum-fed calves, with most deaths occurring during the first week of life (Smith and Little, 1922; Crowley et al., 1994; Wells et al., 1996).

Oral colostral supplements and replacers are available and can be offered when either the dam's or some other fresh or frozen colostrum source is not available; however, the calf small intestine is unable to absorb intact IgG molecules after approximately 26 h of age (Stott et al., 1979a). Once the period of intestinal permeability to immunoglobulin molecules has passed, passive immunity can be provided by intravenous, intraperitoneal, or subcutaneous injection (Anderson et al., 1987; Crowley et al., 1994; Quigley and Welborn, 1996). Calves

provided with an intravenous infusion of a bovine plasma product containing 47 g IgG have increases in serum IgG of approximately 2.9 g/L (Quigley and Welborn, 1996).

The effect of initial IgG concentration at the time of intravenous administration of a concentrated bovine plasma product on post infusion IgG concentrations has not been examined. Therefore, the objective of this study was to determine the effect of different levels of passive immunity on calf response to administration of intravenous immunoglobulin **(IVIG)**.

MATERIALS AND METHODS

Dairy bull calves (n = 32) that weighed between 36 to 50 kg were obtained immediately after birth and assigned to one of four treatments. Calves in the high colostrum group (H) and the control group (C) received 2 L of pooled colostrum (**MC**) at 1 h and 12 h after birth. Calves in the low colostrum group (L) received 1 L of MC mixed with 1 L of milk replacer (**MR**) at 1 h and 12 h after birth. Finally, colostrum deprived calves (D) received 2 L of MR at 1 h and 12 h after birth. Pooled colostrum was collected prior to the start of the study and frozen in 2 L aliquots. Colostrum was thawed in warm water prior to feeding calves at 1 h and 12 h of age. All feedings were administered to the calf via esophageal feeder. Calves were housed indoors in individual pens for the duration of the project and were offered 2 L of milk replacer two-times daily starting at 24 h of age. Water was available ad libitum, but no calf starter or hay was offered during the trial.

Calves in groups H, L, and D received 500 ml of a concentrated plasma product (IVIG) administered through a jugular catheter at 3 d of age. Control calves were administered 500 ml of 0.9% NaCl instead of IVIG at 3 d of age. All infusions were administered slowly over approximately 20 minutes. The IVIG used in this trial was concentrated from bovine abattoir

blood to a final concentration of approximately 35 g IgG/L. Blood was collected from all calves by jugular venipuncture just prior to infusion and again at 24 h post infusion for IgG determination by turbidimetric immunoassay (Etzel et al., 1997).

Experimental data were analyzed using the general linear models procedure of SAS (1996). Orthogonal contrasts were used to test differences between H and C, IVIG and saline, and H and D. Because of the death of one calf prior to 3 d of age, least squares means are reported. Significance was declared at $P < 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Mean plasma IgG concentrations at 3 days of age (Table 5.1) were different between H, L, and D calves, but not between group H and C calves. This was expected since calves in group H and C received colostrum containing 48 g/L IgG, while calves in group L received a mix of colostrum and MR providing 21 g/L IgG. Calves in group D received MR only. All IgG concentrations for calves in group D were below the detection level of the assay (< 1 g/L) and were assumed to be zero.

Table 5.1. Least squares means of treatment parameters for calves with different levels of passive immunity.

	Treatments					Contrasts		
Item	H	L	D	C	SE	1	2	3
No. calves	8	8	8	7	...	...	...	...
Colostral IgG, g/L	48.2	21.0	0.0	48.2	...	...	...	...
Plasma IgG, g/L								
Pre-infusion	12.11	6.25	0.00	11.79	0.71	NS[3]	0.001	0.001
Change (post-pre)	1.83	2.39	3.02	-0.08	0.36	0.001	0.001	0.03

[1]H = 2 L of MC at 1 h and 12 h after birth, IVIG on d 3; L = 1 L of pooled MC mixed with 1 L of MR at 1 h and 12 h after birth, IVIG on d 3; D = 2 L of MR at 1 h and 12 h after birth, IVIG on d 3; C = 2 L of MC at 1 h and 12 h after birth, 0.9% NaCl on d 3
[2]Contrasts: 1 = H vs. C; 2 = IVIG vs. 0.9% NaCl; 3 = H vs. D
[3]$P > 0.10$

Administration of IVIG resulted in a greater increase in mean IgG concentration at 24 h post-infusion compared to calves receiving 0.9% NaCl. Calves in groups H, L, and D all had post-infusion increases in IgG concentration, while calves in group C showed a slight decrease in IgG concentration.

The increase observed in plasma IgG concentration in calves from group D is slightly higher than observed by Anderson et al. (1987) and is likely a reflection of differences in total IgG administered. Calves in the current experiment received concentrated bovine plasma containing approximately 35 g/L IgG, whereas calves in the trial conducted by Anderson et al. (1987) received bovine plasma for which the IgG content was not reported. Immunoglobulin G concentration of adult bovine plasma is approximately 11.2 g/L for IgG_1 and 9.2 g/L for IgG_2; however, values are even lower during colostrogenesis in the dairy cow (Butler, 1986; Guy et al., 1994). Therefore, it is likely that the content of the IgG in the infused product used in that experiment was much lower than in the current study.

Calves in group D had a greater increase in IgG concentration post IVIG infusion compared to calves in group H, suggesting that initial IgG concentration can affect calf response to IVIG administration. Administration of IVIG increased IgG concentrations from 12 to 13.8 g/L in H calves and from 0 to 3 g/L in D calves. Immunoglobulin G concentrations above 14 g/L are commonly reported in colostrum-fed calves, with values above 20 g/L reported for calves allowed to suckle (Stott et al., 1979b; Stott et al., 1979c). Therefore, it is unlikely that an upper threshold for IgG was reached in H calves after administration of IVIG. It is also possible that high levels of maternal antibody in H calves increased the destruction or elimination of infused IgG. Besser et al. (1988) reported that approximately 1.5% of infused IgG is excreted daily in the feces, and 2.5% is excreted daily in the urine.

However, colostral feeding level did not affect excretion of infused IgG in that trial. Our data does not appear to support this previous research in that calves with high levels of passive immunity appear to have a higher disappearance of infused IgG compared to colostrum deprived calves.

CONCLUSIONS

The results from this study indicate that intravenous administration of 500 ml of a 35 g IgG/L product to calves at 3 d of age can increase plasma IgG concentrations regardless of the calf's initial IgG concentration. This can be beneficial in instances when initial IgG concentration is not known, and the health status of the calf is questionable. However, the increase in plasma IgG concentration after IVIG administration is greater in calves with lower initial IgG concentrations. Further research is needed to determine the mechanism resulting in high initial IgG concentration affecting calf response to administration of IVIG.

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CHAPTER SIX

CHARACTERIZATION OF REACTIONS TO INTRAVENOUS IMMUNOGLOBULIN IN NEONATAL CALVES

A paper to be submitted to Veterinary Immunology and Immunopathology

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Abstract

Intravenous immunoglobulin (IVIG) products improve passive immunity in neonates. Unfortunately, adverse reactions can occur. This study was designed to determine if physiological changes occurring after IVIG administration were the result of rapid infusion of large molecular weight molecules or from a more complex mechanism resulting in histamine release. The IVIG was concentrated from bovine abattoir blood and contained approximately 35 g IgG/L. A dextran (75,000 MW) solution was prepared as a high molecular weight control that was similar in osmolarity to the IVIG. Holstein bull calves (n=15) under 1 wk of age were assigned to one of three treatment groups: control calves received 500 ml of 0.9% NaCl; dextran calves received 500 ml of dextran; IgG calves received 500 ml of IVIG. Treatments were rapidly administered (less than 5 min) intravenously via jugular catheter. Heart rate, respiration rate, and blood pressure were measured prior to treatment, and at 1, 3, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min after start of infusion. Blood samples were obtained at the same sampling times, centrifuged, and the plasma immediately placed on ice for determination of histamine concentration using an enzyme immunoassay. Mean respiration rates were higher in calves treated with IVIG

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compared to calves in the other two groups at all time periods measured. Mean heart rates were lower in calves treated with IVIG compared to calves in the other groups through 30 min. Calves treated with dextran tended to have higher mean heart rates than calves on the control treatment from 10 min through 30 min. Mean blood pressure tended to be higher in calves treated with IVIG compared to calves on the control treatment at 1 min, however, there were no differences between groups at any other time period. Mean histamine concentrations were higher in calves treated with IVIG compared to calves on the control treatment at 1 min and were higher in calves treated with dextran at 5 min. Histamine concentrations were not different between groups at any other time period. These data indicate that adverse reactions to IVIG in calves are not mediated by high molecular weight molecules or by histamine release.

Abbreviations: FPT, failure of passive transfer of immunity; IVIG, intravenous immunoglobulin; PAF, platelet activating factor

Introduction

Calves are essentially agammaglobulinemic at birth, although a small percentage of calves may have negligible amounts of circulating serum IgG_1, IgG_2, IgM, and IgA. In ruminants, passive immunity is provided through ingestion of colostrum by the neonate after birth (Smith and Holm, 1948; Klaus et al., 1969). Colostrum is vital to the health and survival of the neonatal calf, and 18% of dairy cows provide colostrum yields with less than 100 g of IgG, the most commonly recommended amount to prevent failure of passive transfer (FPT) (Levieux and Ollier, 1999). Colostrum-deprived calves are 50-75 times more likely to die before 21 d of age than colostrum-fed calves, with most deaths occurring during the first week of life (Smith and Little, 1922; Crowley et al., 1994; Wells et al., 1996).

Oral colostral supplements and replacers are available and can be offered when either the dam's or some other fresh or frozen colostrum source is not available; however, the calf small intestine is unable to absorb intact IgG molecules after approximately 26 h of age (Stott et al., 1979). Once the period of intestinal permeability to immunoglobulin molecules has passed, passive immunity can be provided by i.v., i.p., or s.c. injection (Anderson et al., 1987; Crowley et al., 1994; Quigley and Welborn, 1996). Intravenous infusions of a bovine plasma product in calves can increase serum IgG approximately 2.9 g/L (Quigley and Welborn, 1996).

Unfortunately, adverse reactions to intravenous immunoglobulin (IVIG) administration can occur and have been reported to occur in approximately 25-55% of foals, a species where IVIG products are commonly used to treat FPT (Brown et al., 1991; Liu et al., 1991; Franz et al., 1998; McClure et al., 2001). The incidence rate for reactions in foals appears to be related to product IgG concentration and method of production, with infusion of lyophilized IgG products resulting in an increased rate of reactions (Franz et al., 1998). Intravenous immunoglobulin products are also commonly used in human medicine to treat a variety of disorders, and adverse reactions are reported to be due to anaphylaxis, complement activation, vasoactive properties of the infusion product, or other unknown mechanisms (Ewalenko and Deloof, 1984; Stangel et al., 1997).

Clinical signs of adverse reactions and mechanism of action causing these reactions have not been documented in calves receiving IVIG. Therefore, this study was designed to characterize adverse reactions to IVIG in calves, and to determine if physiological changes occurring after IVIG administration were the result of rapid infusion of large molecular weight molecules or were due to a more complex mechanism resulting in histamine release.

Materials and methods

Animals and treatments

Fifteen Holstein bull calves under one week of age were assigned to one of three treatment groups. Treatment groups were: (1) Control, calves received 500 ml of 0.9% NaCl; (2) Dextran, calves received 500 ml of a dextran solution; and (3) IgG, calves received 500 ml of an IVIG product. The dextran solution was prepared by adding 10 g of dextran powder (75,000 MW) to 500 ml of a NaCl solution. The dextran solution was prepared so that the osmolarity of the solution (269 mOsm) was similar to that of the concentrated plasma product (277 mOsm). The IVIG product was prepared from plasma obtained after a two-time enrichment process of bovine abattoir blood, with the final product containing approximately 35 g IgG/L. Previous analysis showed that the IVIG product had low endotoxin levels and low complement activity.

Preparation and sample collection

Catheters were aseptically placed in each jugular vein so that the treatment could be administered through one catheter, while blood samples were simultaneously obtained from the other catheter. A catheter was also placed in an ear artery and connected to a manometer for arterial blood pressure monitoring.

Calf heart rate, respiration rate, and mean arterial blood pressure were measured prior to the start of treatment infusion, and then at 1, 3, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 min after the start of the infusion. Blood samples were collected into tubes containing EDTA at the same sampling times, centrifuged, and the plasma immediately placed on ice for determination of histamine concentration.

Histamine assay

The amount of histamine present in plasma was determined using a commercially available ELISA (Immunotech, Marseille, Cedex 9, France).

Statistical analysis

Experimental data were analyzed using the general linear models procedure of SAS (SAS Inst. Inc., Cary, NC). Multiple measurements recorded over time were analyzed using the mixed procedure of SAS with calf within treatment interaction used as the random statement. Significance was declared at $P < 0.05$ and trends towards significance between $P > 0.05$ and $P < 0.10$.

Results

Two calves died within 30 minutes after the infusion of IVIG, therefore, they were excluded from the results, and least squares means are reported. There was an overall treatment effect ($P < 0.001$), an overall time effect ($P < 0.001$), and a time by treatment effect ($P < 0.001$) for respiration rate (Figure 6.1). Mean respiration rates were not different among treatments prior to the start of infusions, but were higher in IgG calves compared to calves in the other two groups at all other time periods measured. Respiration rate also showed a large increase after the start of the infusion in calves receiving IVIG, whereas respiration rate remained more or less constant in calves on the other two treatments.

There was no overall treatment effect for heart rate (Figure 6.2); however, there was a time effect ($P < 0.001$) and a time by treatment effect ($P < 0.001$). Mean heart rates were not different among treatments prior to the start of the infusions; however, heart rates decreased in calves receiving IVIG, and were lower compared to calves in the other two groups through 30 min post infusion. Heart rates increased in both control and dextran calves, and dextran

calves tended to have higher mean heart rates compared to control calves from 10 min through 30 min post infusion.

Figure 6.1. Mean respiration rates for calves receiving saline, dextran, or intravenous immunoglobulin.

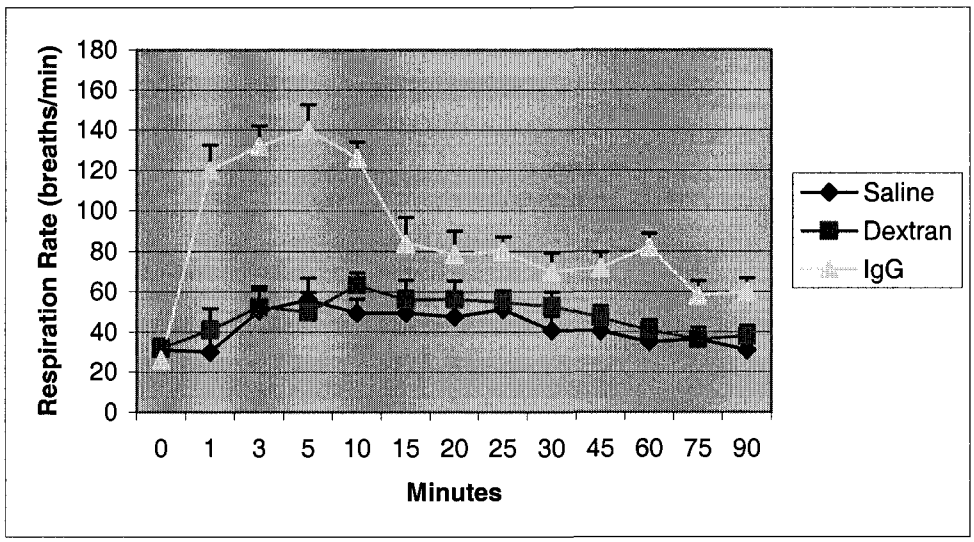
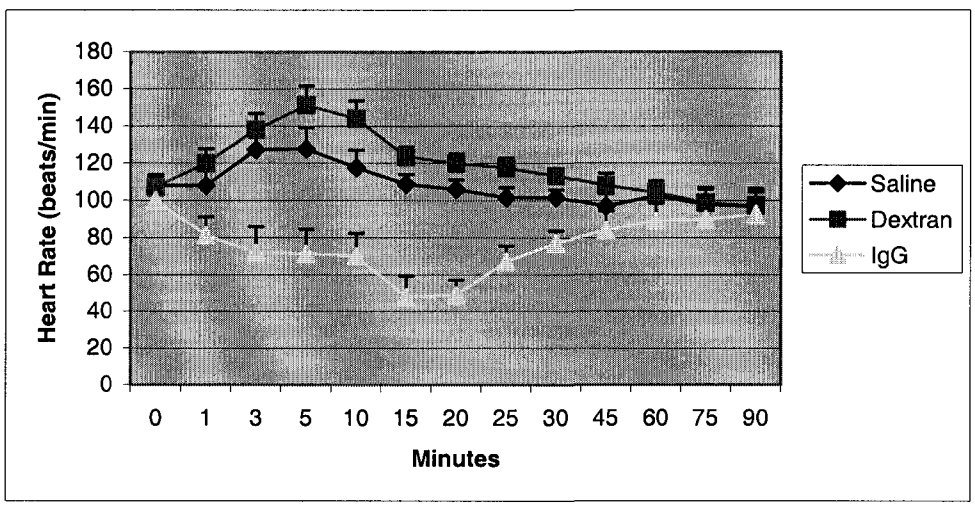


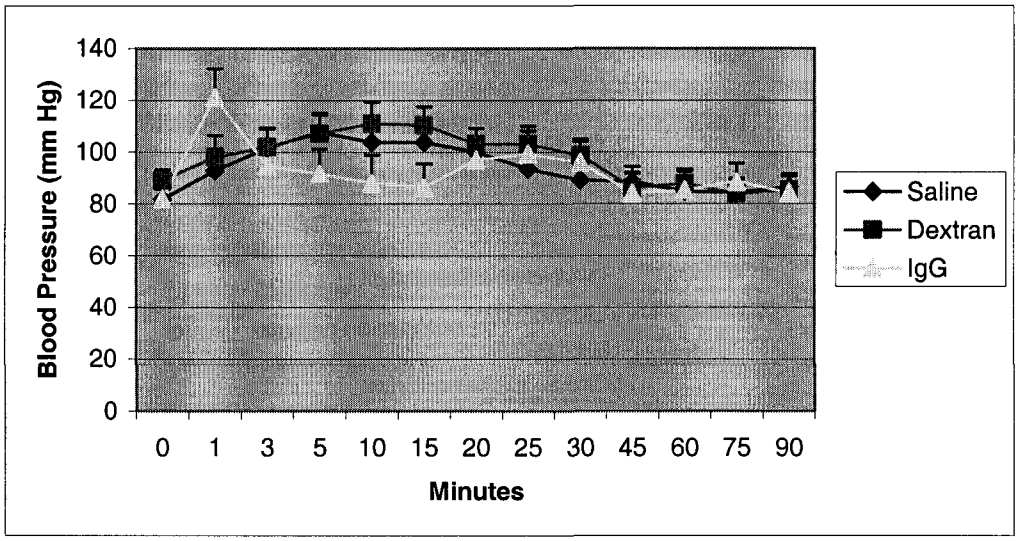
Figure 6.2. Mean heart rates for calves receiving saline, dextran, or intravenous immunoglobulin.



There was no overall treatment effect for mean arterial blood pressure (Figure 6.3), however there was a time effect ($P < 0.001$) and there tended to be a time by treatment effect ($P < 0.07$). Mean arterial blood pressure tended to be higher in claves treated with IVIG

compared to the other two treatments at 1 min post infusion; however, mean arterial blood pressure was not different between treatment groups at any other time period measured. After the start of the infusion, blood pressure increased initially and then decreased rapidly in IgG calves. In contrast, calves receiving the other two treatments had a more gradual increase and subsequent decrease in arterial blood pressure.

Figure 6.3. Mean arterial blood pressure for calves receiving saline, dextran, or intravenous immunoglobulin.



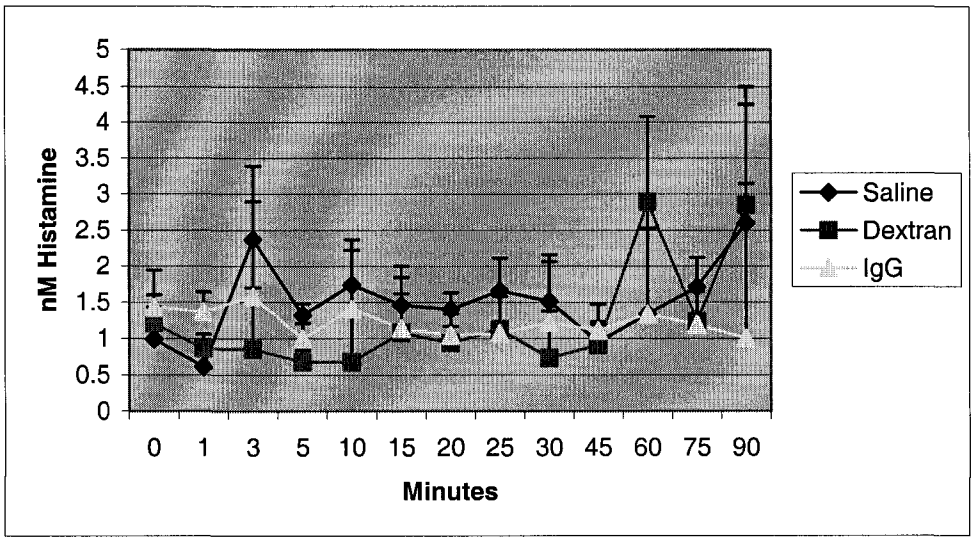
Mean histamine concentrations (Figure 6.4) were higher in calves treated with IVIG compared to calves in the control treatment at 1 min and were higher in calves treated with dextran at 5 min. Histamine concentrations were not different between treatment groups at any other time period measured.

Discussion

The IVIG product utilized in this trial consistently caused obvious clinical signs of infusion reactions in all calves. Within seconds after the start of the infusion, calves showed increases in respiration rate and became increasingly dull and lethargic. In contrast, calves

receiving the dextran and saline solutions showed no visible clinical signs of discomfort, although they did show increases in heart rate. The increase in heart rate is likely due to expansion in fluid volume, and could be attributed to the rapid infusion of large molecular weight molecules in the dextran group.

Figure 6.4. Mean histamine concentration for calves receiving saline, dextran, or intravenous immunoglobulin.



There are three ways by which exposure to a substance can cause anaphylaxis: 1. exposure to a foreign protein that results in IgE antibody formation. Reexposure results in IgE mediated degranulation of mast cells and basophils; 2. formation of immune complexes that activate the complement cascade; and 3. administration of certain agents (hyperosmolar solutions, radiocontrast agents, etc.) that directly stimulate the release of mediators by unknown mechanisms (Bochner and Lichtenstein, 1991). Classic anaphylactic reactions are defined as resulting from a Type I immune response, also called immediate hypersensitivity. This type of reaction requires three components: 1. an antigen; 2. IgE antibody; and 3. effector cells such as mast cells and basophils that synthesize and release pharmacologic

mediators (Ewalenko and Deloof, 1984; Carlson et al., 1986). Reactions that appear clinically similar to anaphylactic reactions, but are not mediated by IgE are referred to as anaphylactoid reactions (Ewalenko and Deloof, 1984; Carlson et al., 1986). Because the calves on this trial did not have previous exposure to IVIG, it is unlikely that IgE mediated the observed reactions. Therefore, the observed reactions in this trial will be classified as anaphylactoid reactions for the remainder of this discussion.

Increased histamine concentrations are often observed in anaphylactic and anaphylactoid reactions. However, some studies report increased histamine levels after challenge in control mice (Choi et al., 1998), while others report no change in histamine levels before or after anaphylaxis in both control and mast cell deficient mice (Jacoby et al., 1984). Hypotension and death due to anaphylactic reactions in mice also do not appear to require mast cell-derived mediators (Martin et al., 1993; Miyajima et al., 1997). Adverse reactions to IVIG in humans have also been shown to be associated with increases in IL-6 and thromboxane B_2 without concurrent changes in blood pressure, kininogen, histamine, or tryptase (Bagdasarian et al., 1998).

All histamine concentrations reported in this study were assumed to be well within the normal range. Plasma histamine concentrations were never greater than 3 nM histamine. Emau et al. (1984) reported resting plasma histamine concentrations of 12 nM in six-month-old calves, and normal plasma histamine concentrations in humans range from 2-8 nM (Morel and Delaage, 1988). Mean whole blood values for histamine in calves at 1 week of age are 173 nM (Wrenn et al., 1963), and whole blood histamine concentrations are generally over 100 times greater than plasma histamine concentrations (Morel and Delaage, 1988).

Because no elevation in histamine was observed in the calves showing severe anaphylactoid reactions, it is likely that the reactions were caused by the release of another mediator such as platelet activating factor (PAF). Incubation with IVIG causes activation of neutrophils and macrophages via Fc receptors resulting in PAF release (Bleeker et al., 1989; Teeling et al., 1998; Bleeker et al., 2000). Rabbits have been shown to release increased amounts of PAF into their plasma within 120 sec after antigen challenge during IgE-induced systemic anaphylaxis (Pinckard et al., 1979). Anaphylactic shock in mast cell deficient mice is suppressed by CV-3988, a specific antagonist to PAF, but not by cyproheptadine, an antagonist to histamine and serotonin (Arimura et al., 1990). Pretreatment with PAF acetylhydrolase or PAF antagonist also blocks fatal anaphylactic reactions in both mast cell deficient and control mice (Choi et al., 1998; Fukuda et al., 2000).

The systemic responses and mortality associated with anaphylaxis are mediated through FcRγ, as mice lacking the FcRγ show no cardiopulmonary changes or mast cell degranulation, and do not die after induction of anaphylaxis. A functional FcεRI is not required for mast cell degranulation or mortality associated with anaphylaxis, as mice lacking this receptor exhibit responses similar to FcεRI +/+ mice (Miyajima et al., 1997). The production of PAF is unrelated to mast cell activation and depends on mononuclear cell activation via FcεRII or FcRγ (Pellon et al., 1993).

Administration of contrast media and hyperosmolar solutions to humans can also cause reactions similar to those observed from infusion of IVIG (Genovese et al., 1996). However, the osmolarity of the dextran and the IVIG solution were both similar to the osmotic pressure in calves. The osmotic pressure in newborn calves is 293 mOsm/L and decreases to 286

mOsm/L after feeding (McEwan et al., 1968). Thus, hyperosmolarity is not the cause of the anaphylactoid reactions observed in these calves.

These data indicate that the adverse reactions to IVIG observed in this trial are not mediated either by high molecular weight molecules or by the release of histamine. Further research is needed to determine the role of platelet activating factor and other potential mechanisms in anaphylactoid reactions in calves.

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CHAPTER SEVEN

GENERAL CONCLUSIONS

The research presented in this dissertation represents a continuing effort to understand colostral supplements and replacers that provide exogenous passive immunity to neonatal animals. The newborn calf and foal are at an increased risk for disease and death if adequate levels of passive immunity are not attained (Boyd, 1972; McGuire et al., 1977; Clabough et al., 1991; Wittum and Perino, 1995). Complicating the ability of the neonate to attain adequate passive immunity levels is the fact that the small intestine of the calf and foal is only able to absorb intact immunoglobulins for approximately 24 h after birth (Jeffcott, 1974; Stott et al., 1979). Exogenous supplements to enhance passive immunity can be given orally provided the neonate is less than 24 h old, or by intravenous injection after that time. The findings from the studies described in this dissertation support the fact that passive immunity can be enhanced through both of these methods.

In the study described in Chapter Two, foals were offered a colostrum supplement at 10 and 12 h of age to provide a total of 24 – 30 g of IgG. All foals were allowed to nurse their dams, and the supplement was tested for its ability to enhance passive immunity in naturally nursing foals. This study showed that providing a colostrum supplement with low IgG concentration does not raise circulating IgG concentrations in naturally suckling foals.

In Chapter Three, a more concentrated oral product for foals was studied that provided a total of 72 g of IgG. In this study, supplement treated foals were muzzled to prevent nursing from their dam through 12 h of age. Treated foals had higher IgG concentrations compared to control foals at 5 h and 48 h of age, and IgG concentrations were not different between the two groups at any other time period measured. This study showed that providing foals with a

colostrum supplement high in IgG soon after birth could result in circulating IgG concentrations similar to or better than that of foals allowed to suckle.

Chapter Four described a study looking at providing newborn calves with colostrum supplements or replacers designed to provide a total of 150 g of IgG. A second concentrated plasma fraction that contained elevated concentrations of IGF-1 and TGF-β was also added to two of the treatment groups. Feeding 150 g of IgG at 1 h of age was superior to feeding 150 g of IgG split in two doses 7 h apart. All calves that received 150 g of IgG in one dose soon after birth had plasma IgG concentrations above the recommended concentration of 10 g/L. However, addition of a plasma fraction containing elevated growth factors decreased plasma IgG concentration. Although the growth factors were concentrated in the serum fraction, concentrations were still well below those found in bovine colostrum (Hadorn et al., 1997; Ginjala and Pakkanen, 1998). It is not possible to determine the exact mechanism responsible for the decrease in plasma IgG, but it can be concluded that elevated levels of growth factors in supplement formulations used in this trial either decreased IgG absorption from the small intestine or increased IgG metabolism.

Although the three trials looking at oral passive immunity products cannot be compared directly, several conclusions can be made. First, products containing low levels of IgG are ineffective for increasing plasma IgG concentrations in neonates that are provided with colostrum. Concentrated IgG products providing 72 g of IgG for foals and 150 g of IgG for calves are effective in raising plasma IgG concentrations above the recommended level needed to prevent failure of passive transfer in these animals. Second, addition of elevated concentrations of IGF-1 and TGF-β to colostrum replacer formulations does not enhance IgG absorption, and in fact appears to either inhibit IgG absorption or increase IgG metabolism.

The final two studies described in this dissertation examined the intravenous use of concentrated bovine plasma (IVIG). Chapter Five was designed to examine the effects of initial IgG concentration on calf response to IVIG. Initial IgG concentration appears to influence calf response to IVIG in that calves with lower initial plasma IgG concentrations have a larger increase in plasma IgG concentration after IVIG infusion. The design of this study did not allow determination of the mechanism responsible for this change. Radiolabeling of the infused IgG molecules would allow half-life of the IVIG to be calculated and excretion rate for the IVIG could be determined.

Unfortunately, some calves exhibit adverse reactions during the infusion of IVIG. Chapter Six was designed to characterize these reactions as they occur, and to also determine if the reactions were related to the rapid infusion of large molecular weight molecules. The incidence rate of adverse reactions to IVIG in humans is often associated with rapid infusion rate (Stangel et al., 1997). Because we wanted to provide the greatest risk for inducing adverse reactions in the calves, all treatments were administered in less than 5 min through a large bore jugular catheter. All calves that received IVIG showed classic signs of infusion reactions including tachypnea and bradycardia, and two calves died within 30 min of the start of the infusion.

Classic anaphylactic reactions are defined as resulting from a Type I immune response and require three components: 1) an antigen; 2) IgE antibody; and 3) effector cells such as mast cells and basophils that synthesize and release pharmacologic mediators (Carlson et al., 1986). Reactions that appear clinically similar to anaphylactic reactions, but are not mediated by IgE are referred to as anaphylactoid reactions (Carlson et al., 1986). The clinical signs of adverse reactions resulting from IVIG infusion in humans are usually associated with the

release of preformed mediators from mast cells and basophils, including the release of histamine (Bochner and Lichtenstein, 1991). Because the calves in this trial would not be expected to have circulating IgE antibodies against the IVIG, we classified the reactions observed in the trial as anaphylactoid. However, because the reactions observed in these calves appeared similar to anaphylactic reactions, elevated histamine concentrations were expected. Surprisingly, there were no differences between treatment groups for mean arterial blood pressure or for plasma histamine concentration. In support of our finding, Bagdasarian et al. (1998) reported adverse reactions to IVIG in humans associated with increases in IL-6 and thromboxane B_2, but without concurrent changes in blood pressure, kininogen, histamine, or tryptase. Unfortunately, the design of the experiment described in Chapter Six did not allow us to answer which mechanism, other than histamine release, accounted for the adverse clinical signs observed in the IVIG calves.

Potent inflammatory mediators resulting from arachidonic acid metabolism, such as platelet activating factor (PAF), prostaglandin E2, thromboxane B2, and leukotriene C4 may be involved in the anaphylactoid reactions to IVIG observed in calves. The release of PAF in mast cell deficient mice leads to clinical signs of anaphylaxis (Arimura et al., 1990). Also, anaphylactic shock in mice is suppressed by PAF acetylhydrolase or PAF antagonists, but not by cyproheptadine, an antagonist to histamine and serotonin (Arimura et al., 1990; Choi et al., 1998; Fukuda et al., 2000). It is clear that if IVIG products are to be made available commercially, further research must be completed in order to eliminate the risk for adverse reactions, or at the very least, minimize their detrimental effects.

In summary, colostrum is vital to the health of the neonate. If colostrum is not available, and the neonate is less than 24 h old, a colostrum replacer providing at least 72 g of IgG for

foals or 150 g of IgG for calves should be provided. When neonates are older than 24 h of age, IVIG can be provided. The increase in circulating IgG concentrations after IVIG administration is small, and therefore multiple doses may be required to raise IgG concentrations to a protective level.

There is a vast amount of knowledge still awaiting discovery in the field of exogenous passive immunity. The half-life of passively administered IgG has yet to be determined. The effects of different manufacturing methods on IgG absorption have been lightly examined, although new and improved methods are always evolving. A more challenging research area deals with optimal IgG concentrations needed to confer disease protection. How does this differ for different pathogens? How does this change with management conditions? Passive immunity is of the utmost importance to neonatal animals until they reach an age where endogenous immunity can adequately protect against disease. Any research that increases our ability to enhance passive immunity will improve health and survivability of the neonates during this vulnerable time.

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APPENDIX

COMPLEMENT FIXATION TESTING OF AN INTRAVENOUS IMMUNOGLOBULIN PRODUCT

Objective

The objective of this experiment was to test the intravenous immunoglobulin product used in Chapter Five and Six for complement activation activity.

Materials and Methods

Sheep whole blood was collected via jugular venipuncture into sterile tubes containing EDTA. The blood was mixed with an equal portion of Alsever's solution and refrigerated until needed. Erythrocyte suspensions were prepared as needed after centrifugation and resuspension in phosphate buffered saline (PBS). Anti-sheep red blood cell (hemolysin) and guinea pig complement were used to determine complement fixation (Colorado Serum Company, Denver, CO). Hank's Balanced Salt Solution (with Ca++ and Mg++) was utilized as the diluent. Blood was also collected from three cows and the serum pooled for use as a complement source in Experiment 1.

EXPERIMENT 1

Experimental Design

Experiment 1 was designed to determine the optimal dilution of hemolysin and complement required to run the assay. All dilutions were run in triplicate, and the microtiter plate was prepared as diagrammed in Table A.1. The same experimental design was also repeated using bovine serum in place of guinea pig complement as the complement source.

Results

The results for guinea pig complement are presented in Table A.2.

Table A.1. Microtiter plate set up for complement fixation Experiment 1

Complement	Hemolysin							
	1:1000	**1:2000**	**1:4000**	**1:8000**	**1:16000**	**1:32000**	**1:64000**	**None**
1:5	25 µl H[1] 25 µl RBC[2] 50 µl D[3] 25 µl C[4]	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
1:10	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
1:20	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
1:40	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
1:80	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
1:160	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
1:320	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	25 µl H 25 µl RBC 50 µl D 25 µl C	0 µl H 25 µl RBC 75 µl D 25 µl C
None	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C	As above except for 25 µl D in place of C

[1]H = hemolysin in 1:4000 dilution
[2]RBC = 0.5% sheep red blood cell suspension
[3]D = diluent
[4]C = guinea pig complement

Table A.2. Complement fixation results for Experiment 1

Complement	Hemolysin							
	1:1000	**1:2000**	**1:4000**	**1:8000**	**1:16000**	**1:32000**	**1:64000**	**None**
1:5	Lysis	Lysis	Lysis	Lysis	Lysis	Lysis	Partial lysis	Partial lysis
1:10	Lysis	Lysis	Lysis	Lysis	Partial lysis	Pellet	Pellet	Pellet
1:20	Lysis	Lysis	Lysis	Partial lysis	Partial lysis	Pellet	Pellet	Pellet
1:40	Lysis	Lysis	Partial lysis	Partial lysis	Pellet	Pellet	Pellet	Pellet
1:80	Lysis	Partial lysis	Partial lysis	Pellet	Pellet	Pellet	Pellet	Pellet
1:160	Partial lysis	Partial lysis	Pellet	Pellet	Pellet	Pellet	Pellet	Pellet
1:320	Partial lysis	Pellet	Pellet	Pellet	Pellet	Pellet	Pellet	Pellet
None	Partial lysis	Partial lysis	Partial lysis	Partial lysis	Partial lysis	Partial lysis	Partial lysis	Partial lysis

No complete lysis was observed when bovine serum was used as the complement source.

Conclusions

Dilutions of 1:20 guinea pig complement and 1:4000 hemolysin are the optimal dilutions to use in this test system. Bovine serum does not work as a complement source at the dilutions tested.

EXPERIMENT 2

Experimental Design

Experiment 2 was designed to test the complement fixation ability of an intravenous immunoglobulin product (IVIG). The IVIG was also tested as a 1:1 dilution as the cloudiness of the product made visualization of results difficult. All dilutions were run in triplicate, and

the microtiter plate was prepared as diagrammed below in table A.3. All columns below the 1:40 complement dilution were filled as described for the 1:40 complement dilution.

Table A.3. Microtiter plate set up for complement fixation Experiment 2

Complement Dilution	**Well Number**			
	1-3	**4-6**	**7-9**	**10-12**
1:40	25 μl H[1] 25 μl RBC[2] 50 μl D[3] 25 μl C[4]	0 μl H 25 μl RBC 75 μl D 25 μl C	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl IVIG[5]	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl dilute IVIG[6]
1:80	25 μl H 25 μl RBC 50 μl D 25 μl C	0 μl H 25 μl RBC 75 μl D 25 μl C	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl IVIG[5]	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl dilute IVIG
1:160	25 μl H 25 μl RBC 50 μl D 25 μl C	0 μl H 25 μl RBC 75 μl D 25 μl C	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl IVIG[5]	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl dilute IVIG
1:320	25 μl H 25 μl RBC 50 μl D 25 μl C	0 μl H 25 μl RBC 75 μl D 25 μl C	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl IVIG[5]	25 μl H 25 μl RBC 25 μl D 25 μl C 25 μl dilute IVIG
None	As above except for 25 μl D in place of C	As above except for 25 μl D in place of C	As above except for 25 μl D in place of C	As above except for 25 μl D in place of C

[1]H = hemolysin in 1:4000 dilution
[2]RBC = 0.5% sheep red blood cell suspension
[3]D = diluent
[4]C = guinea pig complement
[5]IVIG = intravenous immunoglobulin product
[6]Dilute IVIG = 1:1 dilution of IVIG with heat inactivated bovine serum

Results

The results are presented in Table A.4.

Conclusions

The addition of IVIG in the test system did not affect complement at a hemolysin dilution of 1:4000.

Table A.4. Complement fixation results for Experiment 2 after centrifugation (1 min x 200g)

Complement Dilution	**Well Number**			
	1-3	**4-6**	**7-9**	**10-12**
1:40	Lysis	Pellet	Lysis	Lysis
1:80	Partial lysis	Pellet	Partial lysis	Lysis
1:160	Pellet	Pellet	Partial lysis	Partial lysis
1:320	Pellet	Pellet	Pellet	Partial lysis
None	Pellet	Pellet	Pellet	Pellet